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CLINICAL AND METABOLIC
ASPECTS OF
FRUCTOSE

CLINICAL AND METABOLIC ASPECTS OF FRUCTOSE

Papers Presented at a Symposium
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FOREWORD

FRUCTOSE — SWEET WITHOUT RISK FOR HEALTH?

OPENING OF THE SYMPOSIUM

Esko A. Nikkilä

Fructose is a natural compound which the mammalian body can both synthesize and metabolize. In an adult organism the former process evidently occurs to a very small extent whereas the machinery intended for the assimilation of exogenous fructose has such a high capacity that even total basic energy consumption can be covered by this single dietary constituent without difficulty. In fact, fructose is utilized by the body much more readily and in a less complicated way than glucose. As the nature seldom equips itself with devices which it does not need one should be ready to believe that fructose is intended to form one major natural nutrient of herbivorous and omnivorous animals including man. This view also implies that fructose — in moderate amounts at least — does not have any adverse effects in the body.

In the form of refined sugar fructose has become a favored nutrient the consumption of which has been steadily increasing during this century. As this progress coincides with spread of many diseases, notably most conspicuously with the increase in the prevalence of atherosclerosis and coronary heart disease,

it is self-evident that sucrose has been placed on the black list of those exogenous factors which may be related to the genesis of these disorders. Even though there is so far little evidence on a causal association of sucrose consumption and atherosclerosis there are in fact several possible ways by which sucrose could promote atherogenesis. It causes prompt increases of blood glucose and plasma insulin, a combination which is thought to stimulate lipid synthesis in arterial wall, adipose cell and liver resulting in atheroma, obesity and hyperlipemia, respectively. Sugar is intended to be used as a sweetener but actually has become a significant source of calories, which often form just the extra calories leading to excessive increase of adipose tissue. Whether a sucrose-rich diet is also involved in the etiology of diabetes remains unanswered but most data do not support this hypothesis. Of course, sucrose may manifest a latent diabetes more readily than other dietary carbohydrates.

The basic differences in the physiology and metabolic influences of fructose and sucrose were recognized already a century ago when Köllz (1874) showed that diabetics can tolerate

fructose much better than sucrose. This early observation was subsequently well confirmed in clinical and experimental studies and it received an adequate explanation when it became clear that fructose neither stimulates insulin release nor needs insulin for its own metabolism in the body. As fructose thus causes no increase of plasma insulin and elevates blood glucose much less than an equivalent amount of sucrose it appears to be devoid of many of the adverse effects of the latter. As fructose furthermore has a good antiketogenic activity it seems to be a useful sweetener for diabetics.

This view is supported also by some controlled clinical trials. However the general opinion of clinicians through years has been against the use of fructose and it has been repeatedly concluded that fructose should not be recommended for diabetics. There is no question that this view is correct for an overweight diabetic to whom no sugar is better than any sugar.

Until recently the use of fructose as an ordinary dietary constituent has been much limited by its high price as compared to sucrose. A few years ago the chemists at The Sugar Company Ltd. succeeded in developing a new manufacturing process for fructose and this has made it possible to prepare fructose in industrial scale at a reasonable price. This of course has been followed by marketing and advertising of fructose as a natural sweetener the use of which is more healthy than that of sucrose. The increasing availability and use of fructose, then, gives

a challenge to both basic and clinical research for further exploration of the metabolic effects, beneficial or harmful, of dietary fructose in a normal man and in different disorders like diabetes, obesity and hyperlipemia. An additional and highly interesting area of research is formed by the interaction of fructose with the metabolism and metabolic effects of ethanol and by the potential therapeutic use of fructose in ethanol induced disorders like ethanol intoxication, hangover and fatty liver. One further question concerns the influence of fructose on caries.

In parallel with the industrial production of fructose some clinical studies on the use of fructose in the diet of diabetics and of patients with hypertriglyceridemia were initiated in Helsinki. It then appeared that in spite of a rather extensive research on the biochemistry and physiology of fructose no recent review article had been published or any conference held on the subject. This gave the idea for the present symposium of Clinical and Metabolic Aspects of Fructose. The Finnish Sugar Company Ltd. was generous enough in sponsoring the symposium in spite of being well aware of the possibility that most data to be presented were not favorable for the sale of this product. The organizers of the symposium were happy to receive positive responses from almost all of the invited speakers and other participants. In opening the symposium I will direct our appreciation to the sponsor and to the lecturers, who also have prepared their manuscripts in good time to allow a rapid publication of this book.

**METABOLISM OF FRUCTOSE AND
HEREDITARY DISORDERS
OF FRUCTOSE METABOLISM**

INTESTINAL ABSORPTION OF SUCROSE

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Abstract Sucrose is synthesized in the green leaves of plants. With increasing economical status the sucrose from sugar cane and beets, like fat, supplies an increasing fraction of our food. Sucrose is easily metabolized and utilized. Too high consumption is, however, not desirable from nutritional point of view since this highly refined product contains calories but no essential nutrients.

The general concept is that animals (and humans) do not synthesize sucrose. A single report of a sucrose-synthesizing patient needs confirmation from other sources before it can be accepted.

The intestinal absorption of sucrose can only occur if the hydrolyzing enzyme, invertase (sucrase), is present in the mucosal cells. Acid hydrolysis in the stomach has been suggested, but does not occur. The intestinal invertase is an α -glucosidase.

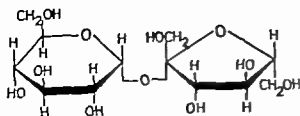
In the human intestine invertase, as well as the other α -glucosidases, is developed very early in fetal life — much earlier than lactase. In the animals studied so far in contrast, intestinal invertase and other α -glucosidases are weak until the weaning period, when lactase disappears and the α -glucosidase develops. The reason for these species differences is yet unexplained.

Human populations with low sucrose consumption have approximately equally high intestinal invertase activity as those in countries with high sucrose consumption. In Greenland Eskimos the adults have for a very long period of time (probably thousands of years) consumed nearly

no carbohydrates at all. The average intestinal activity of invertase and the other α -glucosidases in the Greenland Eskimos is nevertheless nearly the same as ours. Specific enzyme defects, which among us occur only as very rare cases of inborn errors of metabolism are, however, rather frequent in the Greenland Eskimos.

From chemical point of view sucrose is β -D-fructofuranosyl- α -D-glucopyranoside (Fig. 1) i.e. it is composed of one molecule of fructose and one molecule of glucose, and each one of the two monosaccharides is joined to the other one through a glycosidic link. Sucrose for this reason has a number of chemical peculiarities. It is non-reducing, and it is in fact stable against boiling also with very strong solutions of alkali. Due to the fructofuranoside part it is, however, much more easily hydrolyzed by acids than is any of the other common disaccharides.

Sucrose is synthesized in the green leaves of probably all plants. It is then stored in the fruits and roots as a source of energy and carbon.



Sucrose

Fig. 1 The chemical structure of sucrose is β -D-fructofuranosyl- α -D-glucopyranoside.

It is not assumed that sucrose can be synthesized in the human body or in animals. However a research group in Moscow (16) some years ago described a patient who was claimed to excrete 100–200 grams of sucrose per day in the urine. This finding was said to confirm reports of a few patients published by German authors in the 1930-ies. No sucrose was found in the blood, and the synthesis of the disaccharide was therefore assumed to occur in the kidneys. The possibility that the patient might deliberately add sucrose to the urine was considered, but still the patient was kept under careful observation to prevent such addition, the urine continued to contain sucrose.

Although this report originates from a research group with good reputation, it seems difficult to accept until similar cases have been described by other groups within a reasonable period of time. To me it appears quite too likely that a patient, who for some reason wants to simulate diabetes, will try to add sugar to the urine. If she does not have elementary knowledge of biochemistry she may believe that the sugar found in the urine of diabetic patients is the same sugar as we use in tea or coffee, and add that kind of sugar. It is also quite likely that the patient may be skilful enough to add sugar to the urine in spite of the doctor's attempts to control this. Urine obtained by catheterization should be analyzed, which does not seem to have been done.

Even if some humans were able to synthesize sucrose, they would hardly be able to compete with our two most important commercial sources of sucrose, namely the sugar cane and the sugar beet, which account for nearly 60 and 40 %, respectively of the total amount of sugar consumed (22).

The amount of sucrose consumed per individual and time, like the consumption of fat, increases when the average income in the country increases. From the official figures it can be calculated that at present the poorest countries, like India, Pakistan and China, consume around 15 g of sucrose a day per individual, and the wealthiest countries, as USA, England, Scandinavia and others, 150 g (22). In the countries with high sucrose consumption sucrose would according to these figures account for nearly one-fourth of the total calorie consumption. This figure is, however probably somewhat overestimated.

From nutritional point of view too high sucrose consumption is not desirable, since it is a highly refined product containing no essential nutrients. High sucrose consumption increases dental caries, and according to the concept of some authors it may also increase the incidence of atherosclerosis. Nevertheless both humans and animals have a strong preference for the sweet taste of sucrose and sucrose-containing foods (22).

Like other disaccharides sucrose must be hydrolyzed into its monosaccharide components (i.e. glucose and fructose) before it can be absorbed in the small intestine. There is no significant intestinal transport of unhydrolyzed sucrose, and in animal experiments we have found that sucrose administered by injection is quantitatively excreted in the urine.

Since sucrose is so sensitive to acid hydrolysis, it has been suggested that the hydrolysis of ingested sucrose might start already in the stomach, being catalyzed by the hydrochloric acid. We have not found any

Table I. Disaccharidases and glycosidases in the small intestine. The brush border enzymes account for the digestion of dietary disaccharides while the soluble and lysosomal enzymes probably have other functions.

A) Brush border enzymes

- 1) Isomaltase This enzyme also hydrolyzes maltose, and accounts for about 80 % of the total maltase activity
- 2) Invertase (= sucrase) Also this enzyme hydrolyzes maltose, and accounts for about 25 % of the total maltase activity
- 3) Heat stable maltase
- 4) Lactase.
- 5) Trehalase.

B) Soluble or lysosomal enzymes

- 1) Acid β -galactosidase.
- 2) Hetero- β -galactosidase
- 3) Acid α -glucosidase

evidence for acid hydrolysis of sucrose in the stomach, however when we studied the digestion and absorption of test meals in humans (8). The hydrolysis of sucrose in the digestive tract seems to be catalyzed by enzymes, as is the hydrolysis of other food components. This concept is also supported by the fact that patients with congenital lack of intestinal invertase (sucrase), are unable to digest and absorb sucrose (2, 13, 21).

The small intestine contains a number of disaccharidases (Table I). These enzymes are localized in the brush borders of the mucosal epithelial cells, and exert their function in this location. They are thus not secreted. The invertase also hydrolyzes maltose and some other α -glucoside substrates. This and other facts show that the intestinal invertase is an α -glucosidase, in contrast to the invertase in yeast which is a β -fructosidase.

The ability of a subject to absorb a specific disaccharide is dependent on the intestinal activity of the enzyme which hydrolyzes the disaccharide in question. It would therefore be of interest to know which factors regulate the activity of the different disaccharidases in the intestine. One apparent possibility is that the presence of disaccharides in the food may stimulate the corresponding disacchari-

dase activity by adaptation. Some such effects have been observed, but it must be stated that in these cases the effect is relatively weak, and in many cases no effect at all of dietary disaccharides on the intestinal disaccharidase activity could be seen. Therefore substrate adaptation in the sense usually meant by this expression is not the most important factor in the regulation of the small intestinal disaccharidase activity. Although in some instances changes in the activity of the intestinal disaccharidases seem to coincide with changes in the composition of the diet, the changes in enzyme activity are not produced by these changes of the diet. There are some rather curious species differences in the development of the small-intestinal disaccharidases, which cannot be explained at present, but which may with time throw some light on the regulating factors. So the intestine of a newborn pig has high lactase activity but low activity of invertase and the other α -glucosidase activities (3, 4, 7, 14). This means if a newborn pig is fed sucrose instead of lactose, it will get diarrhea, and eventually die. During and after weaning the lactase will decrease, and the α -glucosidases increase (Fig 2). These changes closely follow the dietary changes. If milk feeding is continued, however the same changes in enzyme activity will nevertheless occur and therefore the enzyme activity is not directly controlled by the diet, but by some other mechanism, for instance hormonal (12).

In the human the development of the disaccharidases is quite different. The α -glucosidases (including the invertase) develop already during early fetal life (5), and are thus fully developed a long time before birth. The lactase, in contrast, develops rather late in the fetal life (Fig. 3). In North Europeans and North-Americans the high lactase activity present at birth persists throughout life, but in most other human populations the lactase activity decreases markedly after the first years of life, and in these populations the adult human (like the adult animals of the

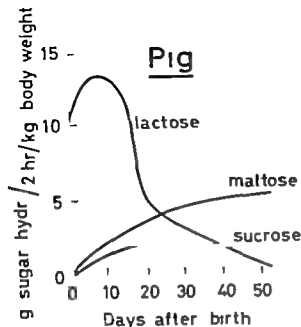


Fig. 2. Development of small intestinal disaccharidases in the pig (3, 14)

mammalian species so far studied) is intolerant of lactose (for review see 10—11). Also in these populations, however, the α -glucosidases seem to develop far before birth.

If we take as an example the development of lactase in human, the activity of this enzyme in the adult subject is not dependent on the dietary habits either of the single subject or of the last few generations of his ancestors. If the dietary habits over a larger period of time are taken into consideration, however, there is a clearcut correlation between the persistence of intestinal lactase in the adult human and the dietary habits of the population to which he belongs (18, 19). This seems to indicate the results of a natural selection in the sense described by Darwin rather than the result of enzyme adaptation.

Rosenzweig et al. (17) have studied the influence of dietary sugars on the small-intestinal enzyme activity by enzyme activity assays in mucosal biopsies. They found no effect of lactose, but sucrose administration increased to some extent the activity of in-

vertase. Administration of fructose had the same effect. I have earlier stated that there is at least a 10-fold variation between different populations in the average sucrose consumption. It would then be of interest to know whether these differences in sucrose consumption are reflected in the mucosal invertase activity. In Table II the mean values for the maltase, invertase, trehalase and lactase activities in one Indian and one Thai group (low sucrose consumption) are compared with the values for two American and one Swedish group (high sucrose consumption) (5, 13, 20). The maltase, invertase and trehalase activities are approximately the same in all these groups. The lactase activity in contrast, is much lower in the Indian and Thai groups than in the American and Swedish groups, which is in accord with our previous knowledge (see Table I). The total carbohydrate consumption is comparable in all these populations, since the low sucrose intake in the Indian and Thai groups is compensated with a higher consumption of starch.

We have also studied the intestinal disaccharidase activities in a group of subjects

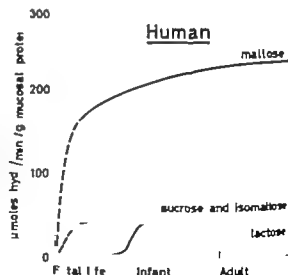


Fig. 3. Development of small-intestinal disaccharidases in the human (9)

Table II. Mean values for disaccharidase activities in populations of high and low sucrose consumption.

Mean values for disaccharidase activities in studies of normal subjects in India (20) and Thailand (13) which represent populations with low sucrose consumption, compared with American controls (subjects newly arrived to India and Thailand, respectively) and a reference group studied in a Swedish investigation (6). The American and Swedish groups represent populations with very high sucrose consumption. The invertase activity appears to be approximately the same in the high-sucrose and the low-sucrose populations. The lactase activity in contrast, is low in the Indian and Thai groups, which is in accord with earlier experiences.

	Units/gram protein (mean)				
	American controls	Indian	American controls	Thai	Swedes
	n=30	n=30	n=23	n=74	n=37
Maltase	263	224	373	292	338
Invertase (= sucrase)	88	81	108	79	69
Trehalase	43	19	—	—	24
Lactase	38	2.4	36	2.1	29

of a population in which the adults for probably several thousands of years have consumed essentially no carbohydrates at all, namely Greenland Eskimos (1). The findings in these are rather astonishing. The mean values of the different disaccharidases are not very much different from those of the other populations studied (maltase 187 units/gram protein, invertase 50, trehalase 14, and lactase 4.8). Out of 19 adult Eskimos studied, 17 were intolerant for lactose, which only shows that, as should be expected, the Greenland Eskimos belong to those populations in which lactase deficiency is the normal state in the majority of the adults. A surprising fact was, however the frequent occurrence of other specific enzyme deficiencies.

In the same group of 19 Eskimos, 3 subjects completely lacked invertase and isomaltase, and 2 subjects showed a nearly complete lack of trehalase. In other populations such specific enzyme deficiencies occur only as very rare congenital defects (inborn errors of metabolism) with a frequency which is probably below 1/10 000. Omission of carbohydrates during a very long period of time from the diet of a population thus does not seem to result in a generally low level of disaccharidase activity in the small intestine, but rather in an increased frequency of specific complete or nearly-complete enzyme defects. It is understandable that in populations in which the adults do not consume carbohydrates such deficiencies do not involve any disadvantages. It is more difficult to find, however any reasons making them more fit to survive.

I have gone into much detail about the regulation of the intestinal disaccharidase activity especially the invertase, and I will therefore have to omit details about some other aspects on the intestinal absorption of sucrose which, however also are very fascinating. I will just mention that there is for instance some yet not fully understood relation between the invertase and isomaltase activities. Although these activities are exerted by different enzyme molecules, these two enzymes seem to be built in together in a common substructure of the microvillous membrane. They also seem to be controlled by a common gene, so if one of the enzymes is absent as the result of a genetic change, the other enzyme is absent too (2). There also seems to be a spatial relationship between the sucrase and a specific membrane carrier for glucose released from sucrose during hydrolysis, giving such glucose a kinetic advantage over free glucose. The carrier is thus different from the carrier for free glucose (6). These data indicate a very delicate organization on the membrane level, which is yet only partly understood. The

absorption of the fructose component of sucrose does not occur via the same carrier as glucose, but recent data indicate an active transport mechanism also for fructose.

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INTESTINAL METABOLISM OF FRUCTOSE

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Abstract The metabolism of fructose by the small intestine can be analyzed in terms of the following scheme: 1) hydrolysis of fructose containing saccharides, especially sucrose; 2) movement of fructose into the intestinal cell; 3) transformation of fructose into glycolytic metabolic intermediates; 4) formation of fructose from glucose via sorbitol; 5) adaptive regulation of fructose metabolizing enzymes; 6) adaptive responses of other enzymes to fructose. The hydrolysis of sucrose is dependent upon the brush border enzyme sucrase which shows an adaptive response to sucrose diets. The entrance of fructose into the small intestine and the intermediary metabolism of fructose is reviewed. Fructose metabolizing enzymes, fructokinase and fructose-1-phosphate aldolase, are regulated by the presence or absence of fructose, folic acid and drugs. Fructose causes adaptive changes in small intestine glycolytic enzymes and decreases the gluconeogenic enzyme fructose-1,6-diphosphatase. Actinomycin D inhibits the adaptive effect of fructose on glycolytic enzymes which suggests that fructose acts via the protein synthetic mechanism.

The small intestine is capable of absorbing fructose from the intestinal lumen so that the bulk of the fructose finds its way to the liver. A smaller proportion of the fructose is metabolized in the mucosal epi-

thelial cells of the small intestine so that the fructose enters into the glycolytic pathway. The metabolism of fructose by the small intestine can be analyzed in terms of the following scheme: 1) hydrolysis of fructose containing saccharides, especially sucrose; 2) movement of fructose into the intestinal cell; 3) transformation of fructose into glycolytic metabolic intermediates; 4) formation of fructose from glucose via sorbitol; 5) adaptive regulation of fructose metabolizing enzymes; 6) adaptive responses of other enzymes to fructose.

The hydrolysis of fructose containing saccharides is a process unique to the small intestine. All of the other processes in the small intestine in many ways are qualitatively similar to the same processes in the liver.

Fructose containing saccharides, especially sucrose (7) are hydrolyzed by the disaccharidase sucrase which is found in the brush border surface particles (21). Sucrase shows an adaptive response in both man and rats. Sucrose diets in both man (35) and the rat (3, 8) and fructose in man (35) increase sucrase activity. Isocaloric glucose diets also increase intestinal sucrase activity but to a lesser degree than sucrose (37). The adaptive increase of sucrase is a relatively slow

process requiring 2-5 days of sucrose feeding (35). We have postulated that this adaptive increase is due to the effect of the dietary substance on the cells in the germinative crypts. As the cells migrate from the crypts and develop into villus epithelial cells the sucrase activity becomes manifest. The appearance of sucrase activity after institution of a sucrose diet coincides with the turnover time of the villus epithelial cells (36). We have utilized the adaptive effect of fructose to increase the sucrase activity in a child with sucrase deficiency and thus increased her tolerance to exogenous sucrose (11).

Fructose enters into intestinal epithelial cells of man and the rat but not by an active transport mechanism (6, 64). The details of fructose movement into intestinal epithelial cells are unknown.

Once in the intestinal epithelial cell fructose is transformed into either fructose-6-phosphate (F-6-P) or fructose-1-phosphate (F 1 P) by the action of hexokinase (52) or fructokinase (1, 4, 10, 47), respectively. In the presence of both fructose and glucose as would occur with the hydrolysis of sucrose, fructose is metabolized preferentially by fructokinase since glucose competes with fructose for hexokinase but not for fructokinase (57). After the administration of fructose to the rat there is accumulation of large amounts of F-6-P, F 1 P and fructose-1,6-diphosphate (FDP) (24, 25, 31).

Fructose-1-phosphate is further transformed into dihydroxyacetone phosphate (DHAP) and glyceraldehyde by means of fructose-1-phosphate aldolase (10, 48, 49). It has been suggested that this activity is contained in the same enzyme molecule that has fructose-1,6-diphosphate aldolase activity since purification of the liver enzyme has not led to separation of the two activities (19, 22, 33). Both F-6-P and DHAP are metabolized via the well known glycolytic pathway. By analogy with the events in the liver it is presumed that in the intestine glyceraldehyde is further transformed into phosphoglyceric acid by

means of a triose kinase and adenosine triphosphate (ATP) (17). Fructokinase and types I and II hexokinases of the rat and guinea pig jejunums have been purified (53).

In the rat as compared to the guinea pig type II hexokinase predominates and there are only very small amounts of types III and IV hexokinases (53). High concentrations of KCl inhibit rat jejunal hexokinase activity (53). The hexokinases account for at least 30 % of fructose phosphorylation (53).

It has been shown that intestinal hexokinase may exist bound to a particulate fraction (50, 53, 54). Perfusion of rat intestine for short periods of time up to 60 min. has shown an increase in soluble and a decrease in particulate hexokinase. It was concluded that the glucose-dependent increase of hexokinase activity in the soluble cell compartment in the intestinal mucosa of starved rats was due to a release of hexokinase from a particulate subcellular structure (28).

Another system for the phosphorylation of fructose has been found in intestinal mucosa. In guinea pig and rat intestinal mucosa a phosphoenolpyruvate (PEP)-dependent monosaccharide phosphotransferase can transform fructose to F 1 P as contrasted with the ATP-dependent systems in which F 1 P, FDP and F-6-P are formed (61). The physiological significance of this PEP-dependent system is not known.

Rat jejunal phosphofructokinase has been isolated and characterized and has been shown to be stabilized by sulfate and inhibited by sodium (20).

Fructose is converted to glucose by rat (21), guinea pig (9, 23) and hamster intestines (53). Guinea pig intestine appears to convert fructose to glucose to a greater degree than rat intestine (23) presumably due to a very low level of glucose-6-phosphatase in rat jejunum (10, 18). Fructose is also metabolized to lactate in rats and guinea pigs (23, 51).

The accumulation of F 1 P that occurs in the intestine after fructose administration (24)

31) may perhaps involve the same mechanisms that occur in the liver. The rate of F 1 P formation from fructose and ATP is about the same as the rate of formation of glyceraldehyde and DHAP from F 1 P. Thus, it would not be expected that F 1 P would accumulate after fructose administration (56). However such is the case. With fructose metabolism there is a decrease in ATP and inorganic phosphate (29-34). The decrease in organic phosphate levels results in increased activity of adenosine monophosphate (AMP) deaminase activity since AMP deaminase is inhibited by inorganic phosphate. The increased activity of AMP deaminase results in a decrease in AMP levels and increased formation of inosine monophosphate which inhibits the action of fructose-1-phosphate aldolase (29-34, 56).

In the intestine glucose can be converted into sorbitol by the action of aldose reductase, a high K_m enzyme (5-59). Presumably the sorbitol can form fructose via the action of sorbitol dehydrogenase. The physiological significance of these reactions in the small intestine is unknown.

The enzymes which metabolize fructose, fructokinase and fructose-1-phosphate aldolase, can be regulated by the presence or absence of fructose, folic acid and drugs. Thus, adaptive mechanisms exist whereby the activities of these enzymes can be regulated. The term adaptation is used to indicate that a change occurs as a consequence of the action of some agent. The term adaptation should not be taken to mean that any specific mechanism of action is implied. In particular adaptation is not synonymous with the term induction which specifically implies the *de novo* biosynthesis of protein. It may be that some adaptive mechanisms operate via an inductive process but not all adaptive mechanisms necessarily operate in this fashion.

A high fructose diet will cause increased activity of fructokinase and fructose-1-phosphate aldolase in both man (44) and the rat

(59). A glucose diet will give lower values of these two enzymes (44, 59). An isocaloric non-carbohydrate diet results in still lower values and fasting gives the lowest values of all (44-59). However the enzyme activities do not disappear but are at their lowest levels with fasting. A sucrose diet increased the activities of fructokinase and fructose-1-phosphate aldolase as compared to glucose and carbohydrate-free diets within 24 hours (46). In two fasting obese patients who were fed 50 g each of glucose and fructose the increase in enzyme activities occurred within 6 hours (45). The effect of diet in increasing rat intestinal hexokinase (2, 53, 54, 62) and fructokinase (62) activities has been reported and confirmed by other investigators.

Folic acid is able to increase both fructokinase and fructose-1-phosphate aldolase activities as well as the activities of other glycolytic enzymes (41). Both dietary carbohydrate and folic acid increase the fructose metabolizing enzymes within two hours with the maximum activities seen at 6-12 hours with elevated activities still present at 24 hours (43). With increasing dosages of folic acid there are increasing values of enzyme activities (43). It is hypothesized that the action of dietary carbohydrate and folic acid are direct actions on the villus epithelial cells because of the short time period involved necessary for the increased enzyme activities to occur after the administration of fructose and folic acid (41).

We have speculated that fructose causes enzyme induction, i.e., *de novo* synthesis of enzyme protein while folic acid initiates protein synthesis (14). Inhibitors of protein synthesis block the action of dietary fructose on glycolytic and folate-metabolizing enzymes (58) indicating that the protein synthetic machinery is ultimately involved. The effect of inhibitors of protein synthesis on fructokinase and fructose-1-phosphate aldolase activities was not tested specifically

however Dietary fructose, however increases glycolytic enzymes and decreases the gluconeogenic enzyme, fructose-1,6-diphosphatase, while folic acid increases all of the glycolytic enzymes and fructose-1,6-diphosphatase. In the scheme of Jacob and Monod (32) the simultaneous induction and repression of enzymes can explain the action of fructose but not the action of folic acid. Folic acid provides two separate cofactors for purine biosynthesis (60) and one might consider that folic acid enhances the biosynthesis of nucleoside triphosphates for messenger RNA synthesis. However there would have to be a corresponding increase in pyrimidine biosynthesis. A folic acid cofactor involves the biosynthesis of deoxyribose thymidine 5' phosphate which is involved in DNA but not RNA synthesis (60). An increased formation of purines could lead to an increase in guanosine triphosphate (GTP) which is intimately involved in protein synthesis at the ribosomal level (26) and in this way might stimulate protein synthesis. We have postulated that the administration of folic acid results in increased formation of formyltetrahydrofolic acid which then enters the initiation and hence the formation of the Embden-Meyerhof pathway enzymes (14, 41). It seems clear that formylmethylenetetrahydrofolic acid is the initiator of protein biosynthesis in micro-organisms. However this has not yet been proven to be true in mammalian systems. It is of interest that folic acid has no effect on jejunal disaccharidases (41). We thus have to postulate that folic acid initiates the synthesis of only some proteins in jejunal mucosal cells. In a patient with jejunal, hepatic and red cell formotransferase deficiency that we have been studying we have found that there is a marked failure of jejunal enzyme adaptation to fructose and folic acid. Formiminotransferase transforms tetrahydrofolic acid and formimino-glutamic acid into glutamic acid and formimino-tetrahydrofolic acid which is

an intermediate in the pathway leading to N^{10} formyltetrahydrofolic acid. Failure of the adaptive enzyme response in the jejunum of this patient who also has a specific defect in folic acid metabolism is consistent with our hypothesis on the mechanism of action of folic acid (15).

We have utilized the adaptive effect of folic acid to increase the fructose-1-phosphate aldolase activities in the jejunum and liver of a child with hereditary fructose intolerance (12). Coincident with the increase in hepatic and jejunal enzyme activities her hepatomegaly and a fatty infiltrate in the liver both disappeared. Whether the increase in hepatic fructose-1 phosphate aldolase activity was sufficiently large to be of clinical significance, however cannot be stated with certainty.

Orally administered sex hormones (estradiol-17 β and testosterone) will increase the activities of several glycolytic enzymes in rat jejunum (36). Male rats respond better to testosterone while female rats respond better to estradiol-17 β (36). In these studies the effect of sex hormones on rat fructose metabolizing enzymes was not tested. In normal adult males and hypogonadal male patients we have found no response of fructose-1-phosphate aldolase and fructose-1,6-diphosphate aldolase to oral or intramuscular (hypogonadal patients only) testosterone even though pyruvate kinase activities increased (27). In one female child and her mother who were being studied for other reasons we found a similar lack of response of fructose-1 phosphate and fructose-1,6-diphosphate aldolases to oral estradiol-17 β (12). It would appear that the specific fructose metabolizing enzymes of the jejunum are not affected by sex hormones. In rats, folic acid deficiency causes a decrease in jejunal glycolytic enzyme activities (16) and prevents the adaptive increase of enzymes to sex hormones (13).

Fructokinase and fructose-1-phosphate al-

dolase activities have been found to be increased in human jejunum within one day after the administration of phenobarbital, 30 mg, four times per day (42). On the other hand, dexamethasone decreases the activities of these same fructose metabolizing enzymes (38).

Ethanol and folic acid are antagonistic in the jejunum. In man ethanol decreases the activities of jejunal fructose metabolizing enzymes while folic acid increases the activities of the same enzymes. When both are administered together the effect of each is inhibited by the other (40). The mechanism of action of ethanol on fructose metabolizing enzymes is unknown but would seem to be related to the folate effect. It has been known for many years that there is some interaction between folic acid and ethanol but the exact relationship has been unclear. The antagonism between folate and ethanol on jejunal fructose metabolizing enzymes brings this relationship more clearly into focus.

Fructose itself can affect various of the glycolytic enzymes. Fructose will increase the activities of rat jejunal fructose-1,6-diphosphate aldolase (39) pyruvate kinase, phosphofructokinase and glycerol-3-phosphate dehydrogenase (55) and decrease fructose-1,6-diphosphatase (35).

Fructose causes similar changes in human jejunal pyruvate kinase, phosphofructokinase and fructose diphosphatase (39). Fructose causes an increase in the activities in rat jejunum of certain of the folic acid metabolizing enzymes (glutamate formiminotransferase, serine hydroxymethyltransferase and methylenetetrahydrofolate dehydrogenase) and a decrease in formyl-tetrahydrofolate synthetase (37). Fructose may cause these adaptive changes in jejunum via the mechanism of depression and repression. Actinomycin D inhibits the adaptive effect of fructose on these enzymes which is consistent with the mechanism of action of fructose *de novo* protein synthesis (38).

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METABOLISM OF FRUCTOSE IN LIVER

Fritz Heinz

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Abstract For liver metabolic pathway for fructose has been described in which the ketosugar is phosphorylated by ketohexokinase to fructose-1 phosphate, which is then converted by liver aldolase to D-glyceraldehyde and dihydroxyacetone phosphate, an intermediate of the glycolytic pathway. D-glyceraldehyde could be oxidized to glycerate. By phosphorylation glycerate becomes an intermediate of the Embden Meyerhof pathway like D-glyceraldehyde if phosphorylated directly by triokinase. The reduction of D-glyceraldehyde forming glycerol, which could be phosphorylated to L-glycerol 3-phosphate is in variance with isotope studies with fructose-6-¹⁴C.

This special metabolic pathway is limited to warmblooded animals and man, because only in the liver of these species ketohexokinase could be detected.

An adaption of enzymes was found in rats, which have had a high fructose diet over three weeks.

The activity of ketohexokinase estimated under optimal conditions and 37°C agrees well with fructose extraction rates found in liver perfusion for rat and in *in vivo* experiments for human liver. The fast catabolism of fructose in human liver in contrast to glucose, is due to higher enzyme levels of ketohexokinase, in contrast to hexokinase and glucokinase. By this high phosphorylation capacity and the low activity of aldolase together with the action of metabolic inhibitors on this enzyme and the equilibrium

on the side of fructose-1 phosphate, fructose-1-phosphate will accumulate if high fructose concentrations were offered. But even under these conditions, the levels of metabolites following fructose-1 phosphate were enlarged.

Enzyme regulations based on the high fructose-1 phosphate and low ATP levels were discussed.

In 1874, Kùlz (37) reported that fructose, in contrast to glucose, is tolerated by human diabetics. Twenty years later first experiments with pancreatectomized dogs were described by Minkowski (34). According to this author glycogen was synthesized in the liver of animals fed on fructose but not on glucose. In 1902, Oppenheimer (37) found in dog livers perfused *in vitro* that the administration of fructose to the perfusion fluid, in contrast to glucose, resulted in a higher production of lactate. Similar results were obtained by Embden and Isaac (7). These authors found that the biosynthesis of lactate from fructose remained unchanged in perfused livers of diabetic dogs; no lactate was produced by the administration of glucose.

Accordingly the early biochemists trying to get more information about carbohydrate

metabolism, recognized the differences between sugars as glucose and fructose. In 1930—1932, Leuthardt and Testa (22 a, b), Cori et al. (4), and Hers (18) found an ATP dependent enzyme in liver phosphorylating fructose to fructose-1 phosphate. In the newer literature this enzyme was called ketokinase or ketohexokinase. Recently this enzyme was purified from rat liver by Sanchez et al. (42) potassium and magnesium were needed in order to obtain optimal activity. Fructose-1 phosphate is converted to dihydroxyacetone phosphate and D-glyceraldehyde by aldolase 1 B which is present only in the liver (8 a, b 25 38). The properties of the enzyme were different from those of the muscle enzyme. Dikow et al. (6) succeeded in obtaining a pure enzyme preparation from the human liver. Dihydroxyacetone phosphate may enter the glycolytic pathway. Metabolism of the D-glyceraldehyde however can be catalyzed by four different enzymes.

The triokinase catalyzes the phosphorylation of D-glyceraldehyde using ATP to D-glyceraldehyde-3-phosphate (13 20 21 23). The aldehyde dehydrogenase (NAD⁺) oxidizes D-glyceraldehyde to glyceric acid (23, 29 39). The latter compound is again phosphorylated via the glycerate kinase and ATP to 2-phosphoglycerate an intermediate of the Embden Meyerhof pathway (28 30). This metabolic pathway was first discussed by Leuthardt et al. (33). In addition, the formation of serin is also possible by the NAD⁺/NADP⁺ dependent dehydrogenisation of D-glycerate to hydroxypyruvate (11 47). A reduction of the glyceraldehyde to glycerol can occur by the action of two different alcohol dehydrogenases. The so-called normal alcohol dehydrogenase (24 48) requires NADH, the other one requires NADPH and was formerly named aldehyde reductase or glycerol dehydrogenase. Thus glycerol is phosphorylated by the glycerol kinase to L-glycerol-3-phosphate (1 46) a compound belonging to the glycolytic pathway. The

reduction of the D-glyceraldehyde to glycerol is not consistent with isotope studies using fructose-6-¹⁴C (19 41). If this labelled ketohexose is administered to rats, a more pronounced radioactivity is found in the carbon atoms number one and six of glucose derived from the hepatic glycogen. Glycerol formed by the reduction of D-glyceraldehyde-3-¹⁴C derived from fructose-6-¹⁴C, is phosphorylated stereospecifically to L-glycerol-3-phosphate-1 ¹⁴C via the glycerol kinase reaction (2). This observation is in agreement with the Three Point Attachment Theory according to Ogston (36).

L-glycerol-3-phosphate-1 ¹⁴C introduces the label to the carbon atoms number 3 and 4 of the glucose molecule. However this labelling was not detectable in the hepatic glucose from animals treated with fructose-6-¹⁴C. Besides the K_m of 3×10^{-3} M of the liver alcohol dehydrogenase using glyceraldehyde is too high with respect to physiological processes.

These possibilities concerning fructose metabolism and its intermediates — as shown in Fig. 1 — do not give any answer to the following questions:

1. Fructose metabolism, is it a common pathway in the liver of all animals and is man?
2. The fructolytic enzymes, are they localized only in the liver? Are these enzymes definitely involved in fructose metabolism? The enzyme levels, are they high enough in order to explain the well known high metabolic rate of fructose in the liver and even so in contrast to glucose?
3. The application of fructose, does it change the concentrations of metabolic intermediates of fructose and glucose? What is the magnitude of these changes?

METHODS

Enzymes were extracted from liver and measured as was previously described (14). D-glyceraldehyde was used as a substrate for aldehyde

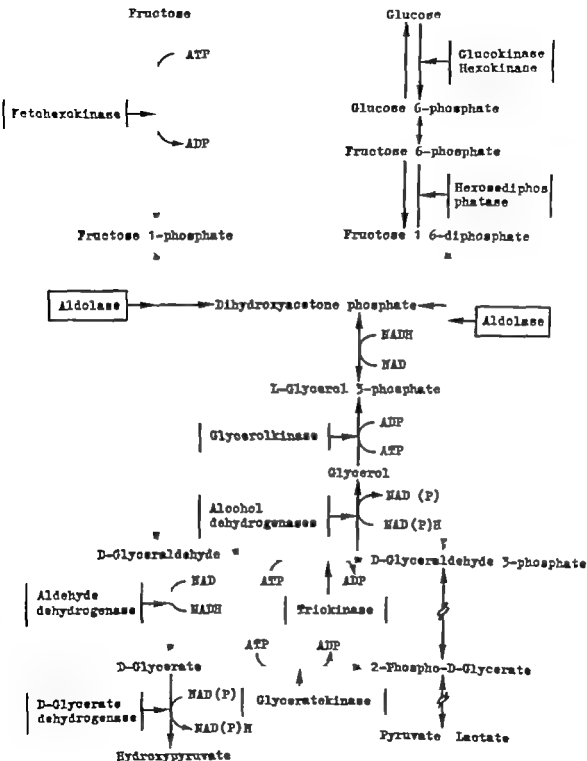


Fig. 1 The metabolism of fructose in liver

metabolism, recognized the differences between sugars as glucose and fructose. In 1950—1952, Leuthardt and Testa (32 a, b), Cori et al. (4), and Hers (18) found an ATP dependent enzyme in liver phosphorylating fructose to fructose-1-phosphate. In the newer literature this enzyme was called ketokinase or ketohexokinase. Recently this enzyme was purified from rat liver by Sanchez et al. (42) potassium and magnesium were needed in order to obtain optimal activity. Fructose-1 phosphate is converted to dihydroxyacetone phosphate and D-glyceraldehyde by aldolase 1B which is present only in the liver (8 a, b, 23, 38). The properties of the enzyme were different from those of the muscle enzyme. Dikow et al. (6) succeeded in obtaining a pure enzyme preparation from the human liver. Dihydroxyacetone phosphate may enter the glycolytic pathway. Metabolism of the D-glyceraldehyde, however, can be catalyzed by four different enzymes.

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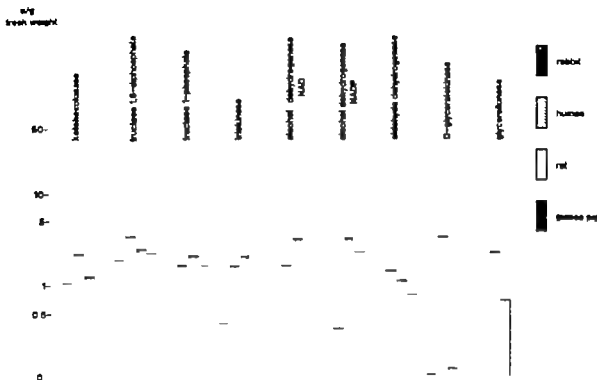


Fig. 3. Activities of fructolytic enzymes in human and laboratory animal liver

ase glyceraldehyde kinase and triokinase, as compared to animals fed on an isocaloric diet containing glucose. The triokinase was most elevated. These differences were also seen if animals were fed on a normal diet. The ketohexokinase was found to be the only exception. These results underlined that the triokinase converting the D-glyceraldehyde to D-glyceraldehyde-3-phosphate is definitely involved in fructose metabolism.

The enzyme levels were determined under optimal conditions and at 25°C. If the activity of the ketohexokinase is transformed to 37°C, the physiological temperature of rats, 3.0 u/g (fresh weight) were found. This value is consistent with the rate of fructose extraction from the perfusion fluid using the perfusion method according to Schlomaker (43). In human liver the ketohexokinase activity is raised to 1.7 u/g at 37°C. This result is in good agreement with the extrac-

tion rate of 1.5–2.3 μ moles fructose/g/min calculated from data obtained in *in vivo* experiments, as was reported by Tygstrup et al. (43) and by Craig et al. (5). It is concluded from these results that fructose is phosphorylated by the ketohexokinase with an optimal rate if the fructose concentration exceeds 20 mg/l.

In rat liver the phosphorylating capacity for glucose is 1.6 μ moles/g/min. This value is similar to ketohexokinase however 1.35 u/g correspond to glucokinase with a high K_m of 1×10^{-3} M and 0.25 u/g correspond to the hexokinase isoenzymes with low K_m values approximating 10^{-4} M. Under physiological conditions with glucose concentrations of 90 mg % in the blood and in the liver only the hexokinase is saturated with this substrate and operates under optimal conditions. The glucokinase phosphorylates about 0.2 μ moles/g/min. We assume that

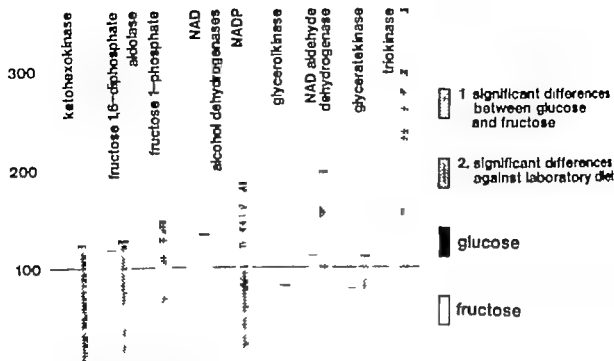


Fig 4 Changes of activities of enzymes involved in fructose metabolism after feeding a diet containing 60% fructose or glucose during 3 weeks. Normal diet = 100%.

these data may explain the observation that in the liver a higher phosphorylating capacity was found for fructose, as compared to glucose. This is more pronounced in the human liver because only traces of glucokinase activity were measured.

By the administration of fructose to rats (75 mg/100 g body weight) by a single and brief injection into the tail vein, the fructose concentration in the blood was increased to 10 μ moles per ml (12). Under experimental conditions, the concentration of fructose-1-phosphate in the liver was as high as 7 μ moles/g fresh weight (Fig 5). The content of ATP is reduced by this high phosphorylation rate (40) and in order to phosphorylate again the ADP the concentration of inorganic phosphate is diminished in the blood (49). The levels of other intermediates as dihydroxyacetone phosphate, fructose 1,6-di-

phosphate, lactate, pyruvate and L-glycerol-3-phosphate were also increased but not as drastically as fructose 1-phosphate. As was demonstrated by Kuppe et al. (26), L-glycerol-3-phosphate derived from fructose is easily incorporated into the liver lipids. Therefore, an augmented supply of this lipid precursor may stimulate lipid synthesis. Our results on fructose intermediates following fructose loading are comparable to those reported by Burch et al. (3).

The accumulation of fructose-1-phosphate may be due to the relative low aldolase activity and its inhibition by fructose-1,6-diphosphate. The latter compound as an intermediate of the Embden Meyerhof pathway is split by the same enzyme. These two criteria and the equilibrium constant between fructose-1-phosphate and dihydroxyacetone phosphate and D-glyceraldehyde which is

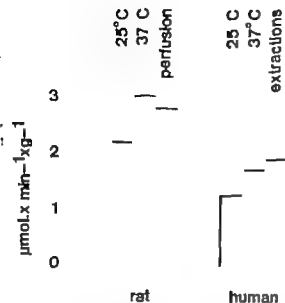
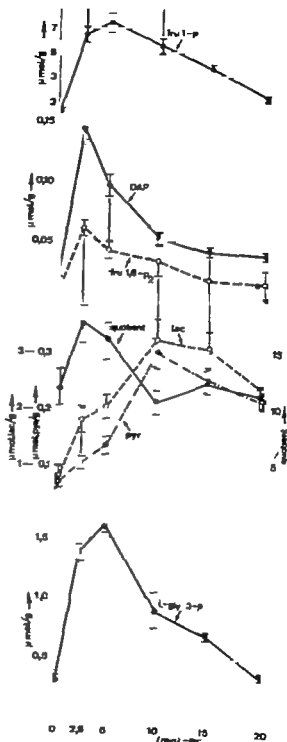


Fig. 5 Comparison of ketohexokinase activity with the extraction rate of fructose from the perfusion media of rat liver and the extraction rate of human liver in *in vivo* experiments.

shifted to about 99 % in direction of fructose-1-phosphate probably lead to an elevation of the fructose-1-phosphate content (31). Besides the accumulation of this sugar phosphate may also be the result of a competitive inhibition of the aldolase by degradation products of the ATP metabolism, as was described by Woods et al. (50). Gluconeogenesis from fructose starting after a lack period when the fructose-1-phosphate concentration decreases, can be inhibited by this product at the stage of the glucose-6-phosphate isomerase (31). Obviously the marked decrease of the ATP concentration and the ratio ATP/ADP (40) during the first phase

Fig. 6 Levels of some metabolites in rat liver after single and brief injection into the tail vein (75 mg fructose per 100 g body weight). fr 1-P = fructose-1-phosphate, DAP = dihy-



droxyacetone phosphate, fr 1,6-p₂ = fructose-1,6-diphosphate, lac = lactate, pyr = pyruvate and L-gly 3-p = L-glycerol-3-phosphate. Quotient = ratio lactate/pyruvate.)

following the application of fructose, changes biochemical reactions requiring energy. As was demonstrated by Sölling (44) the administration of fructose to rats caused a transformation of the pyruvate dehydrogenase to the active dephospho-enzyme in the liver. This activation of the enzyme and the elevated pyruvate concentration increases the acetyl-CoA level as was reported by Helmreich et al. (17). In addition, the formation of the phosphorylase a — the active form — requires ATP therefore, the rate of glycogenolysis may be inhibited. Simultaneously glycogenolysis may be impaired by a diminished supply of inorganic phosphate (49). The system synthesizing glycogen appears to be more complicated. It should be expected that a low ATP concentration would lead to an enhanced conversion of the glycogen synthetase b, the inactive form, to the glycogen synthetase a, the active form. The previous step, however the condensation of the glucose-1-phosphate with UTP forming UDP glucose, depends on a sufficient energy supply within the cell. The reason is that the rephosphorylation of UDP as a product of the glycogen synthetase reaction is coupled to the ATP system.

ACKNOWLEDGEMENT

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FRUCTOSE METABOLISM IN ADIPOSE TISSUE

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Abstract. The rate limiting step of fructose metabolism in dipose tissue is fructose transport into the cell. The step is accelerated by insulin only in the absence of glucose, so that insulin has no effect on fructose transport under physiologic conditions. The fructose carrier has relatively high apparent K_m for fructose transport so that significant quantities are transported only at relatively high concentrations of fructose in the blood.

Fructose is the phosphorylated to fructose-6-phosphate by the enzyme hexokinase. Glucose does not compet because it is not present intracellularly in sufficiently high concentrations. This statement is correct only for adipose tissue.

Fructose uptake by adipose tissue of diabetic animals is reduced as is glucose uptake but to lesser extent.

In vivo, most of the C^{14} -incorporation of fructose- C^{14} into triglycerides or $C^{14}O_2$ by dipose tissue occurs after hepatic conversion of C^{14} fructose to C^{14} -glucose. Insulin is required for glucose transport into adipose tissue.

The main pathway of fructose is its phosphorylation by fructokinase in the liver

small bowel and kidney to fructose-1-phosphate, the splitting of fructose-1 phosphate by the B-aldolase and the subsequent phosphorylation of glyceraldehyde by triosekinase (for review see 5). As we will hear in other papers during this Symposium, fructose phosphorylation occurs very rapidly and the half life of fructose in man is approximately 15 minutes. The utilization of fructose by other tissues has been a matter of controversy for many years. There can be no doubt that fructose can, indeed, be metabolized by some tissues other than liver, small bowel and kidney. Thus, patients with hereditary fructose intolerance who lack the specific hepatic fructose pathway excrete only between 10-20 % of the intravenously administered fructose in the urine (8). The same holds true for patients with essential fructosuria who are deficient in fructokinase (15) the first enzyme of the specific hepatic fructose pathway. Therefore, some tissues of the body must be able to metabolize fructose by other metabolic routes.

THE IMPORTANCE OF FRUCTOSE TRANSPORT INTO VARIOUS TISSUES FOR ITS METABOLISM.

Hexokinase of many tissues phosphorylates fructose at a rate equal to that of glucose. The main difference in the enzyme reaction towards glucose and fructose lies in the K_m of the reaction. Hexokinase of adipose tissue phosphorylates glucose with a K_m between 10^{-4} and 10^{-5} M, whereas fructose is phosphorylated with a K_m between 10^{-3} and 10^{-2} M (3, 10).

Let us consider as a model cells in which glucose and fructose concentrations equilibrate rapidly with the surrounding medium, such as erythrocytes and leucocytes. Red and white blood cells metabolize fructose and glucose to approximately the same extent at concentrations around 200 mg/% in the incubation medium when either one of these sugars is present in the medium. Glucose oxidation is not influenced by the addition of cold fructose to the medium, which is to be expected since the K_m of hexokinase is much lower towards glucose than towards fructose. However if one looks at $^{14}\text{CO}_2$ formation from ^{14}C fructose an inhibition of this process is observed upon adding cold glucose to the incubation medium (Fig. 1). It is, therefore, to be expected that all tissues and all cells devoid of fructokinase which contain free glucose within the cytoplasm do not metabolize fructose although fructose may be transported into the cell because the hexokinase reaction towards fructose is competitively inhibited by glucose. Cells in the body which contain free glucose within the cytoplasm are usually insulin independent with regard to glucose transport. This is certainly true for the above mentioned cells in blood and for the brain and also for liver, small bowel mucosa, proximal tubular endothelial cells and for blood vessels. It is therefore, to be expected that red and white blood cells and brain cells do not utilize fructose under normal circumstances, where-

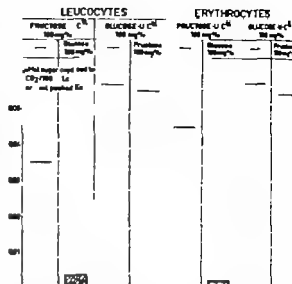


Fig. 1 Fructose and glucose metabolism of leucocytes and erythrocytes. Erythrocytes were separated from leucocytes and incubated in a medium containing ^{14}C -fructose alone or with cold glucose in one set of experiments and ^{14}C glucose alone or with cold fructose in another set of experiments. $^{14}\text{CO}_2$ production was measured. Fructose and glucose metabolism by these cells are similar when only the labelled sugar is present. Cold glucose drastically decreases ^{14}C -fructose oxidation, whereas cold fructose has no effect on ^{14}C -glucose oxidation. (From ref. 8)

as the other mentioned cells utilize fructose by way of the specific fructose pathway rather than by phosphorylation through hexokinase.

The question whether the muscle utilizes fructose or not is still a matter of debate. Again, in *in vitro* experiments, the muscle takes up fructose readily incorporating it into glycogen and oxidizing it to CO_2 (Fig. 2) (4). However when cold glucose is added in increasing amounts to the incubation medium, fructose utilization by muscle decreases. At a concentration of 200 mg % of glucose and in the presence of insulin, fructose utilization is almost completely abolished. This would tend to indicate that there is no free glucose present in the cytoplasm of the cell at concentrations of glucose below 50 to 100

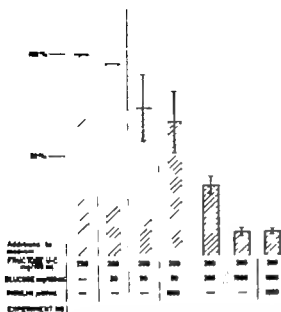
INCORPORATION OF FRUCTOSE- ^{14}C INTO GLYCOGEN AND CO_2 BY RAT DIAPHRAGM

Fig. 2 Fructose metabolism of striated muscle. Rat diaphragm was incubated with ^{14}C fructose alone and with increasing concentrations of glucose with or without insulin. Fructose metabolism is quite considerable when glucose is absent. Increasing glucose concentrations and insulin inhibit the metabolism of fructose. (From ref. 4).

mg /4, whereas free glucose appears to accumulate when insulin acts on higher glucose concentrations, thus inhibiting fructose phosphorylation at the hexokinase level (4, 15). If fructose is administered intravenously to rats together with anti-insulin serum, there is practically no incorporation of ^{14}C into diaphragm glycogen, indicating that fructose metabolism of muscle is probably very limited due to competitive inhibition by glucose (9).

FRUCTOSE AND GLUCOSE TRANSPORT INTO ADIPOSE TISSUE

In this respect, adipose tissue behaves in a completely different manner. Adipose tissue is the tissue of unlimited energy stores in

the form of triglycerides. People with high caloric intake and low caloric output deposit their excess of calories almost exclusively in adipose tissue. On the other hand, adipose tissue fulfills the second most important goal of supplying the starving organism with free fatty acids from which up to 70 % of the energy is derived during fasting. Since adipose tissue does not have major energy requirements other than those needed for fat synthesis and fat mobilization, it can be looked upon as a sack of fat which needs the information of whether or not to take up glucose and to convert it to fat or to release free fatty acids from the triglyceride stores. In order to fulfill this task properly it needs to be practically impermeable to glucose in the basal state. This is, indeed, what one finds (2, 6). Adipose tissue *in vitro* will take up small amounts of glucose even in the absence of insulin, whereas adipose tissue *in vivo* does not appear to take up appreciable amounts of glucose if insulin is altogether lacking (W 13). Glucose membrane transport is therefore the rate limiting step of glucose metabolism. This has been directly proven in very elegant experiments by Crofford and Renold (2). They incubated adipose tissue *in vitro* at 37 °C with high concentrations of glucose and insulin and failed to detect any free glucose in adipose tissue cells. Only when the hexokinase activity was decreased by lowering the temperature to 17°C instead of 37 °C there was actual accumulation of free glucose under the influence of insulin in the tissue. It would appear that the transport of glucose was relatively less affected by the low temperature than the hexokinase reaction so that the latter now became rate limiting. Under physiological circumstances, however this does not seem ever to be the case, and transport of glucose is always rate limiting. We had postulated this many years ago on the basis of experiments carried out with ^{14}C -fructose with and without glucose and insulin (6, 10). It was found that glucose did

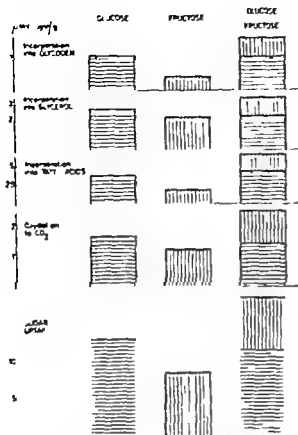


Fig. 3 Additive metabolism of fructose and glucose by adipose tissue. Pooled epididymal rat tissue was incubated in the presence of ^{14}C -glucose (left), ^{14}C -fructose (middle), as well as with both sugars together (always in a concentration of 200 mg/l) with ^{14}C -glucose or ^{14}C -fructose alternately (right). The results of ^{14}C -fructose were added to those of ^{14}C -glucose when both sugars were present in the medium to obtain total hexose metabolism (right column). The results show clearly that fructose metabolism is not inhibited by glucose and vice versa. (From ref. 6).

not inhibit ^{14}C -fructose uptake, oxidation and incorporation into total lipids of adipose tissue. Even in the presence of insulin, glucose and fructose were taken up by the tissue simultaneously and metabolized in an additive manner (6) (Fig. 3). It can, therefore, be concluded that fructose is transported into adipose tissue by a different carrier than glucose and that glucose does not prevent fructose phosphorylation because it is not

present in the free form in sufficient quantities to compete for phosphorylation at the hexokinase level.

Since transport is the limiting step of sugar metabolism by adipose tissue one can deduce useful information on the characteristics of the carrier mechanism by measuring total sugar metabolism by the tissue. Since about 70 % of the glucose or fructose taken up by adipose tissue is metabolized to CO_2 , and total lipids, these two metabolic indices were taken to represent sugar uptake. Whereas glucose uptake of adipose tissue *in vitro* increases slowly when the glucose concentration in the medium is raised, fructose metabolism is very much dependent on the fructose concentration (4, 13). The apparent K_m of glucose transport in the absence of insulin was estimated to be on the order of $4 \times 10^{-4} \text{ M}$ whereas the apparent K_m for fructose was approximately $4 \times 10^{-3} \text{ M}$ (4). Both sugars do not compete for the same carrier site on the membrane under basal conditions if no insulin is present. However fructose uptake is also stimulated, although to a much smaller extent than glucose uptake, by insulin. This insulin stimulation of fructose uptake is completely inhibited in the presence of glucose. It would, therefore, appear that under basal conditions fructose is transported by an insulin independent carrier system but that the insulin effect on fructose uptake occurs by way of the glucose carrier (Fig. 4).

THE ENZYMES OF FRUCTOSE METABOLISM IN ADIPOSE TISSUE

Fructokinase and fructose-1-phosphate aldolase are not present in adipose tissue (10). In all likelihood fructose is phosphorylated by hexokinase to fructose-6-phosphate and hence enters directly the glycolytic pathway. Since the apparent lack of an enzyme in a homogenate does not prove that the respective enzyme is also absent in intact tissue, other evidence for or against direct phospho-

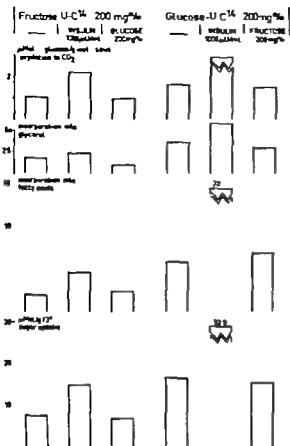


Fig. 4. Mutual independence of glucose and fructose metabolism of adipose tissue and stimulation by insulin. Pooled epididymal rat adipose tissue metabolizes almost as much fructose as glucose at hexose concentrations of 200 $\text{mg}\%$ in the medium. The effect of insulin on fructose uptake is small compared to glucose.

rylation of fructose to fructose-6-phosphate was searched for. Two approaches were used. Adipose tissue was incubated with fructose-1- ^{14}C , glycogen isolated, hydrolyzed and C 1 recovered as $^1\text{CO}_2$ after oxidation of glucose by bacteria. 98 % of ^{14}C in the glucose molecule was present in the C 1 position, indicating that the fructose molecule was not split to trioses and recondensed to fructose-1,6-diphosphate prior to incorporation into glycogen (10). The interpretation of these data has been rendered difficult by the failure to find fructose-1,6-diphosphatase activity in

adipose tissue (17). If this latter enzyme were really missing, then one would pick up no ^{14}C from trioses since their recondensation is impossible. That would then mean that all fructose going to glycogen in adipose tissue must be incorporated after direct phosphorylation of fructose to fructose-6-phosphate. This can, indeed, be concluded from comparative studies on the incorporation of glucose- ^{14}C and fructose- ^{14}C , respectively into glycogen of adipose tissue. Although the relative amount of glucose and fructose taken up by adipose tissue which is incorporated in glycogen may not be the same for both sugars, one does, nevertheless, find a certain relationship between sugar uptake and glycogen synthesis for both sugars. Therefore, fructose appears to be directly phosphorylated to fructose-6-phosphate and to share the same fate as glucose which enters glycolysis as glucose-6-phosphate. Insulin enhances glycogen formation from ^{14}C -glucose to a relatively greater extent than from ^{14}C -fructose (7). Since fructose and glucose use different carriers to enter the adipocyte, these metabolic differences may be due to the compartmentalisation of the adipocyte.

THE METABOLIC FATE OF FRUCTOSE IN ADIPOSE TISSUE

Transport into the cell rather than enzyme activity determines fructose and glucose metabolism of adipose tissue to a major extent. However fructose like glucose metabolism is modulated to some degree by the activity of the triglyceride lipase. When triglyceride lipase is stimulated, free fatty acids are produced and α -glycerophosphate originating from the hexose will be used for the partial reesterification of free fatty acids. Therefore, lipolysis stimulates the incorporation of ^{14}C -fructose into glyceride-glycerol of total lipids. Lipolysis is stimulated by insulin-antagonistic hormones such as glucagon,

Table I. Glucose and fructose transport characteristics in adipose tissue of normal, acutely and subacutely diabetic rats

Pooled epididymal adipose tissue was incubated with 3 different concentrations of either ^{14}C -glucose or ^{14}C fructose (30 mg%, 200 mg% and 800 mg%). The results of $^{14}\text{CO}_2$ and total lipids were added together and taken as a measure of hexose transport. (From ref. 4)

Rats	Glucose Transport into Adipose Tissue		Fructose Transport into Adipose Tissue	
	K_m moles/liter	V_{max} μ moles/g/hr	K_m moles/liter	V_{max} μ moles/g/hr
Normal fed	4.7×10^{-3}	4.5	4.1×10^{-3}	11.5
Acutely alloxan-diabetic, insulin withdrawal	3.0×10^{-3}	2.5	3.0×10^{-3}	7.3
Subacutely alloxan-diabetic, no insulin	4.0×10^{-3}	1.5	9.3×10^{-3}	1.5

adrenaline, ACTH and growth hormone. All these hormones increase the uptake of glucose by adipose tissue to some extent (1-14). Relatively more glucose is oxidized in the tricarboxylic acid cycle whereas the pentose phosphate shunt is not activated by these hormones. No similar experiments have been carried out with fructose. Since the uptake of fructose rather than enzyme activities determine how much fructose is being metabolized, it is likely that the lipolytic hormones have similar effects on fructose as on glucose metabolism.

Thus, overall fructose metabolism depends in the first place on the fructose concentration. Insulin in the presence of cold glucose leads to a relatively increased incorporation of ^{14}C of fructose into fatty acids, whereas lipolytic hormones stimulate incorporation into glyceride-glycerol (7).

FRUCTOSE METABOLISM OF ADIPOSE TISSUE FROM DIABETIC ANIMALS

Fructose and glucose metabolism of adipose tissue of acutely and chronically diabetic rats have been compared in a rather extensive study (4). Glucose and fructose uptake decreased in a parallel manner 48-72 hours after insulin withdrawal in alloxan-diabetic rats. Hexose uptake is still normal when

expressed on a weight basis, but is clearly smaller when expressed per fat pad or per rat. At this early stage of insulin deficiency the response to insulin *in vitro* is unimpaired. At a later stage (120-144 hours after alloxan administration) fructose and glucose uptake of adipose tissue are very much decreased and so is the insulin response of the tissue. This is demonstrated by the results in Fig. 5. In diabetic adipose tissue membrane transport remains rate limiting for glucose and fructose metabolism. The apparent K_m of glucose and fructose transport does not change whereas there is a 3-fold decrease of the apparent V_{max} of glucose transport and a 7 fold decrease of the V_{max} of fructose transport (4) (Table I). Interestingly the two different carrier mechanisms appear to be altered in a similar manner by insulin deficiency. These findings indicate that insulin deficiency leads to a loss of both glucose and fructose carrier. This is in keeping with the results of very interesting experiments which suggested that insulin may stimulate the formation of glucose carrier in adipose tissue membranes (12). The carrier system which is left in diabetic adipose tissue appears to have similar affinities for fructose and glucose as that of normal tissue. Insulin lack seems to reduce the quantity of the carrier rather than change its quality.

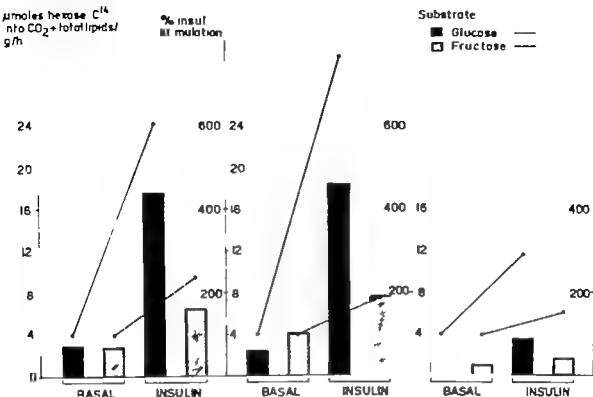


Fig. 5. Fructose and glucose metabolism by pooled epididymal adipose tissue from normal (left), acutely diabetic (middle) and chronically diabetic rats (right). Glucose and fructose metabolism and their response to insulin expressed per g of tissue are not reduced in rats which have been deficient in insulin for 48–72 hours (middle). Basal uptake and insulin sensitivity are markedly reduced after 120–144 hours of insulin lack (right). (From ref. 4)

acids by adipose tissue of normal rats at high rates of hexose uptake, diabetic tissue incorporates less than 20% into fatty acids at maximal rates of hexose uptake. The decreased hexose transport appears to be primarily responsible for the impaired fatty acid synthesis by diabetic adipose tissue.

FRUCTOSE METABOLISM OF ADIPOSE TISSUE IN VIVO

The metabolic fate of glucose and fructose is similarly affected in diabetic adipose tissue (Fig. 5). The percentage of hexose taken up by adipose tissue which is incorporated into fatty acids increases in a parallel manner with the hexose uptake, whereas incorporation into glyceride-glycerol decreases conversely. Since hexose uptake of diabetic adipose tissue is drastically reduced, fatty acid synthesis is minimal. A high percentage of the ^{14}C of hexose is incorporated into glyceride-glycerol. Whereas up to 70% of hexose carbon-14 is incorporated into fatty

The investigation of the direct utilization of fructose by adipose tissue in vivo is rendered difficult by the rapid conversion of fructose to glucose. Thus, when one tries to trace the fate of carbon 14 from injected ^{14}C -fructose one cannot distinguish between direct incorporation of ^{14}C fructose on one hand and the incorporation of ^{14}C -glucose deriving from labelled fructose on the other hand. From our *in vitro* results we dare to extrapolate to the *in vivo* situation and are pretty certain that insulin *in vivo* will not stimulate

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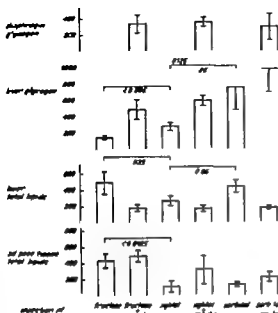


Fig. 7 Fructose metabolism by adipose tissue of streptozotocin-diabetic rats *in vivo*. The metabolism of ^{14}C fructose was compared with that of ^{14}C xylose and ^{14}C -sorbitol. These two polyols are not directly taken up by adipose tissue but rather after conversion to ^{14}C -glucose. The greater ^{14}C incorporation into total lipids seen after ^{14}C fructose administration indicates that fructose is taken up as such by adipose tissue *in vivo* (From ref 9).

want to make here is the fact that fructose is being utilized by adipose tissue *in vivo* independently of insulin, whereas xylose and sorbitol are utilized only after hepatic conversion to glucose and under insulin stimulation.

CONCLUSIONS

Adipose tissue takes up fructose in a concentration dependent fashion. At a concentration of 100 mg /%, fructose and glucose uptake by adipose tissue are equal. Fructose transport is stimulated by insulin only in the absence of glucose. Fructose appears to be transported by a carrier which is distinctly different from the glucose carrier.

The metabolism of fructose by adipose tissue depends mainly on the rate of entry

into the cell which in turn depends on the concentration in the medium. Adipose tissue *in vivo* plays a minor role in the removal of glucose from the blood even in the presence of insulin. The role of adipose tissue consists in the unlimited storage of carbohydrate and fat in the form of triglycerides on one hand and in the provision of free fatty acids as a source of energy replacing glucose.

Adipose tissue is not very important in the overall metabolism of either glucose or fructose *in vivo*. However fructose leads to increased reesterification of free fatty acids, so that their level in the blood decreases which in turn leads to increased glucose oxidation. In contrast to glucose, insulin is not necessary for this effect of fructose.

Adipose tissue of diabetic animals shows a decrease of glucose as well as of fructose uptake *in vitro* and a relative loss of insulin sensitivity. *In vivo* considerable incorporation of ^{14}C -fructose takes place in adipose tissue of streptozotocin-diabetic rats. Fructose decreases lipolysis more markedly than insulin alone by providing more α -glycerophosphate for reesterification.

Fructose has proven to be an extremely interesting sugar with regard to the elucidation of regulatory processes in adipose tissue the most important of which are sugar transport on one hand and the lipolytic activity on the other hand.

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CONTROL OF HEPATIC FRUCTOSE-METABOLIZING ENZYMES FRUCTOKINASE, ALDOLASE AND TRIKINASE

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Abstract. Physiological factors which may regulate the catalytic activity of three key enzymes of hepatic fructose metabolism, fructokinase, aldolase and triokinase, are reviewed. Possible relationships between these potential control mechanisms and hepatic fructose metabolism *per se* are discussed; three distinct levels: 1) characterization *in vitro* of purified enzyme preparations 2) variation of enzyme activity *in vivo* under various dietary and hormonal conditions and 3) variation of enzyme activity *in vivo* following development of liver tumors of different growth rate and degree of cellular differentiation.

The ability of an organism to adapt to environmental change is the basis for the maintenance of a dynamic state in normally functioning cells. Such adaptation requires tight control and regulation of specific enzyme-catalyzed reaction rates. Whereas the focus of past biochemical research was on delineation of broad pathways, in recent years emphasis has shifted towards regulatory mechanisms of specific metabolic pathways and their constituent enzyme-catalyzed reactions. It is the purpose of this article to

review certain aspects of the metabolism of fructose in such a light.

A host of experiments *in vivo* and *in vitro* has established the liver as the most important organ of fructose metabolism, accounting for up to 85 % of the orally administered sugar (for example, see 23 32, 38, 45 67 74). The main results of earlier investigations concerning the metabolism of fructose in liver indicate that it is not introduced as a hexose into the main stream of general carbohydrate metabolism, but only after cleavage into 3-carbon units (45 48 51). Furthermore, there is a small number of acknowledged enzymes which have a specific significance for hepatic fructose metabolism: fructokinase, which phosphorylates fructose in the 1-position; an aldolase which cleaves fructose-1-phosphate (F 1 P) to dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde (GA) and perhaps triokinase, which phosphorylates GA, forming glyceraldehyde-3-phosphate (GAP). Where applicable this review will deal with control of these hepatic enzymes at the following levels of organization: 1) characterization *in vitro* of purified preparations 2) susceptibility *in vivo* to nutritional

and hormonal manipulation and 3) extent of altered genetic expression in hepatic tumors of varying growth rate and degree of cellular differentiation.

CHARACTERIZATION OF HEPATIC FRUCTOSE-METABOLIZING ENZYMES

Fructokinase Liver fructokinase was first examined by Leuthardt and Testa and by Kuyper both of whom demonstrated its broad specificity showing that it phosphorylates L-sorbose, D-tagatose, L-arabinose, L-galactoheptulose and D-altrioheptulose, as well as D-fructose to form the corresponding 1 phosphate (cf. ref. 26).

Fructokinase activity in the liver is substantially higher than that of hexokinase (68) and the former enzyme has a great affinity toward fructose according to Hers (24) the Michaelis constant is about 0.5 mM. Although fructose is readily phosphorylated in liver by hexokinase to fructose-6-phosphate (F-6-P) its low activity and its K_m for fructose of 2 to 6 mM (15, 24) as compared with a K_m for glucose of 0.1 mM (68) makes it unlikely that F-6-P is formed in appreciable quantities under normal circumstances.

F 1 P was indicated as the reaction product in liver by several investigators (12, 33, 36) and finally was verified by Leuthardt and Testa (cf. ref. 76) who obtained the ester in analytically pure form by means of a purified enzyme. According to Hers (26) an ester with the properties of F 1 P is formed also when muscle extracts are incubated with large quantities of fructose.

Although the hexokinases of yeast, brain and muscle are able to react with fructose as well as with a whole series of additional sugars to produce the corresponding 6-phosphate these enzymes possess a very much lower affinity for fructose than for glucose (11, 61). Thus the reaction with fructose is strongly inhibited by glucose. In consideration of the great importance of muscle car-

bohydrate metabolism, the behavior of the muscle enzyme toward fructose is of great interest. According to Colowick (11), at a concentration of 0.25 mM the enzyme is 94 % saturated with glucose, but only 5 % with fructose. This explains why the conversion of fructose in muscle is very slight under physiological conditions. The need of a specific fructokinase that is not inhibited by glucose was made comprehensible from this condition (13). If the liver contained only one hexokinase of the type of the yeast, muscle or brain enzyme, because of the high glucose concentration, there must be little if any utilization of fructose.

Requirements by fructokinase for K^+ and Mg^{++} were demonstrated by several workers (25, 42, 67), and it was shown that the Mg^{++} concentration must equal or exceed the concentration of ATP in order to attain maximal activity. In fact, excess ATP was shown to be so strongly inhibitory that the reaction velocity was reduced to zero when the ATP to- Mg^{++} ratio reached a value of five.

Much greater fructose phosphorylation was demonstrated under aerobic than under anaerobic conditions (67, 69). After Slein (59) showed that the decreased fructose consumption by rat liver homogenates under anaerobic conditions was not due to limiting ATP Parks et al. (42) explored the possibility of product inhibition. They ruled out F 1 P as the inhibitor because addition of fluoride, while resulting in a large accumulation of F 1 P did not inhibit fructose utilization (12, 60).

By adding creatine phosphate and purified muscle ATP creatine phosphotransferase (30) in nonlimiting amounts to the liver fructokinase assay system, ADP could be converted to ATP as rapidly as it was formed. By thus preventing the accumulation of ADP they not only enhanced fructokinase activity but also achieved a proportionality between the amount of enzyme and fructose disappearance, thus providing a useful assay for ad-

in purification (42). They concluded that the effect of aerobic conditions on fructokinase activity was due to removal of ADP presumably by oxidative phosphorylation to ATP. Although fructokinase had been purified only 10 to 20-fold previously (37) using this assay method Parks et al. (42) developed a reliable procedure for purifying beef liver fructokinase 150-fold.

The striking inhibition of fructokinase activity by ADP and excess ATP could well play an important role in the regulation of hepatic fructose metabolism. Although inhibition by excess of substrate is not rare the unique aspect of this particular case is that in the Michaelis constant region slight variation of ATP or Mg^{++} concentrations may have relatively great effects on the reaction velocity. The obvious conclusion is that cellular enzyme control may normally be exerted by alteration of adenine nucleotide or Mg^{++} concentrations associated with various physiological processes.

Recently new spectrophotometric and radiochemical assay procedures for measuring fructokinase activity were reported (1). This enzyme subsequently was purified from the liver of adult rats by acid and heat treatment, ammonium sulfate fractionation, and chromatography on Sephadex G-200 and diethyl aminoethyl cellulose. The enzyme preparation has specific activity of 15 to 30 units per mg of protein at 25° and was purified approximately 1 000-fold from the 100 000 \times g supernatant. Purified liver fructokinase has a K_m of 0.2 to 0.5 mM for fructose and 1 to 2 mM for magnesium adenosine triphosphate, and the enzyme reaction produces equimolar amounts of F 1 P and ADP. Enzyme phosphorylates D-fructose with ATP, 2-dATP or 3-dATP and also phosphorylates L-sorbose, D-xylulose, and L-galactoheptulose with ATP. It is stimulated by cysteine and other thiol, inhibited by p-chloromercuribenzoate and has an activation energy of about 11 kcal per mole and a Q_{10} of 2.

Similar purification and characterization of this enzyme, including more extensive studies on the role of K^+ were reported by Sanchez et al. (52, 53).

Aldolase. In liver F 1 P is immediately cleaved into DHAP and D-GA (37). It is now well established that the predominant, if not the only aldolase of liver differs from the muscle aldolase in composition (50) and in its physical, kinetic, and immunochemical properties (8, 14, 16, 43, 50, 54, 63). Although it had been claimed that the aldolase activities of liver toward fructose 1,6-diphosphate (FDP) and F 1 P were due, respectively to different species of enzymes (30) there seems little doubt from studies of several investigators that a single enzyme species can account for activities toward both substrates. If the muscle type aldolase is present in rabbit liver it must be less than 1 % of the total as determined immunochemically (48). Several of those differences described above, as well as others, recently were confirmed using homogeneous preparations of the liver enzyme (9, 20, 21). These results leave hardly any doubt that liver contains only a single species of aldolase that is responsible for the activity toward both substrates.

Thorough kinetic studies of liver and muscle aldolase were recently completed by Spolter et al. (63), who demonstrated that liver aldolase differs from both native and carboxypeptidase-treated muscle aldolase at the binding site of the substrate to the enzyme. Among several inhibitors of both liver and muscle aldolase that were discovered, the most significant were the adenine nucleotides. Muscle aldolase was competitively inhibited by $ATP > ADP > AMP$. Moreover the ATP inhibition was reversed by Mg^{++} . On the other hand, liver aldolase was competitively inhibited by $AMP > ADP$ but not at all by ATP. It is not inconceivable that these inhibitory actions play a physiological role in controlling the activity of aldolase *in vivo*. As a further

result of these kinetic studies, the Michaelis constant for FDP and F 1 P of liver aldolase was demonstrated to be 0.004 mM and 2 mM, respectively and of muscle aldolase 0.014 mM and 7.0 mM, respectively

Triokinase. The further fate of D-GA formed by aldolase in liver is not yet firmly established. Three possible sequences may be envisioned 1) reduction to glycerol and subsequent phosphorylation to α -glycerophosphate 2) oxidation to glyceric acid with its subsequent phosphorylation and 3) direct phosphorylation to GAP. Although it is possible that under appropriate conditions each of the three above-mentioned routes can be used, the existence of a specific triokinase in liver capable of directly phosphorylating D-GA to GAP implies that this step may be the chief route by which D-GA formed from fructose is converted to glucose. Of the potential GA-utilizing enzymes in liver only triokinase will be considered in this article.

According to Hers and Kusaka (26, 27) there does exist in the liver a kinase which is able to phosphorylate D-GA directly. By ammonium sulfate fractionation these authors obtained from guinea pig liver a protein fraction which produced triose phosphate on F 1 P on incubation with D-GA. The phosphorylation of GA was not competitively inhibited by glycerol in this mixture. These observations established the existence in liver of a kinase specific for GA, denoted as triokinase.

Lindberg (37) first demonstrated that GA can be phosphorylated to GAP in dialyzed kidney extracts. Hers and Kusaka (27) suggested that in crude extracts, rich in triose phosphate isomerase and aldolase, the GAP thus formed would isomerize to DHAP and then react with a second molecule of GA to form F 1 P. Triokinase also was partially purified by ammonium sulfate treatment, and was shown to phosphorylate at approximately equal rates, D-GA, D,L-GA and dihydroxyacetone.

Heinz and Lamprecht (22) reported the preparation of triokinase from beef liver is about 50-fold enrichment. They proposed that triokinase phosphorylated the primary hydroxyl group of D-GA and dihydroxyacetone, yielding the corresponding triose phosphate and ADP. L-GA, D-glycerate, glucose and fructose were not found to be substrates. The Michaelis constant for D-GA was 0.125 mM, for dihydroxyacetone 8 mM and for D,L-GA 0.33 mM. Optimal triokinase activity was demonstrated at pH 7.0.

Hers (28) reported the purification of triokinase (free of glycerokinase) from guinea pig liver and subsequently discovered that ITP cannot replace ATP as a phosphate donor and that optimal activity occurred between pH 7.2 and 7.5.

Although addition of isobutyramide to impure reaction mixtures, to inhibit NADH-dependent reduction of GA by alcohol dehydrogenase facilitated development of a simple, spectrophotometric assay procedure (2) attempts to purify and to characterize this enzyme have been largely unsuccessful.

NUTRITIONAL AND HORMONAL REGULATION

Although rates of fructose utilization have been determined in liver slices from fasted (78) adrenalectomized (4) and alloxan-diabetic (10, 40) rats, relatively little information is available concerning dietary and hormonal effects on the enzymes involved in hepatic fructose metabolism, other than those described by Adelman et al. (3). Marked changes were observed in total liver activity of fructokinase, aldolase and triokinase when rats were maintained under various dietary and hormonal conditions. In contrast specific activities in terms of liver weight did not change greatly.

All three activities decreased to 50% or less of their normal total activity on fasting.

for 48 to 72 hours, and were restored completely in 24 hours by fructose feeding. With glucose feeding, recovery was complete for aldolase but only partial for fructokinase and triokinase. No recovery of activity was detectable following administration of a high protein diet to fasted rats. Long term feeding of fructose resulted in the maintenance of a considerably higher level of all three enzymes over that on a high fat or high protein diet.

These enzyme activities were unchanged in alloxan-diabetic rats. Fructokinase activity of fed adrenalectomized rats was at the fasting level of normal rats and was neither lowered further on fasting nor increased by subsequent feeding of fructose or glucose. In contrast, aldolase and triokinase activities were normal and decreased sharply on fasting, but did not recover on subsequent glucose or fructose feeding. Essentially the same pattern was found for hypophysectomized as for adrenalectomized rats.

The action of fructose in the recovery of fructokinase and triokinase activity after fasting provides additional examples of substrate induction of phosphotransferases, analogous to that of glucose on hepatic glucokinase (36). However the action of fructose on triokinase probably is indirect, by virtue of its metabolic conversion to D-GA (36). The induction of aldolase activity by both glucose and fructose was not unexpected, inasmuch as this enzyme is also required for glucose catabolism. However the decrease in this enzyme activity on fasting was surprising since substrate induction in the opposite direction might have been anticipated in consequence of the increased gluconeogenesis that occurs in this condition. The lack of responsiveness to dietary protein distinguishes these adaptations from those resulting from general protein repletion (39), or those due to induction of specific enzymes of amino acid metabolism (34). Although the long and short term feeding experiments suggest that fructose acts as an enzyme

inducer similar effects are displayed also by glucose, and the activity fluctuations accompany changes in liver weight. Also, fructose feeding apparently can increase the activities of hepatic enzymes not associated directly with fructose metabolism and has no effect on others (18-19). These observations argue against a highly specific substrate induction. On the other hand, the rise in liver weight on fructose feeding is not due to a general increase of protein, but rather to a large increase of liver glycogen.

The absence of any effect of alloxan diabetes on the activities of all three enzymes implies that they are not affected by lack of insulin. This is in accord with previous observations that the utilization of fructose and its conversions to glucose, CO_2 , and glycogen are unimpaired in liver slices of diabetic rats (10-40-47). It is evident that the adrenal glands are required for adaptation to dietary fructose and that these responses may be mediated via glucocortical steroids. The lack of responsiveness to fructose feeding as well as the low levels of activity of these enzymes in hypophysectomized rats may also be attributable to lack of glucocorticoid secretion, since these deficiencies were largely corrected by hydrocortisone administration.

Despite the marked fluctuations in activity described briefly above the enzyme levels were still sufficiently high to account for known rates of fructose metabolism in intact cells. According to Spiro and Hastings (62) liver slices incubated aerobically at 37 until fructose at a rate of 240 $\mu\text{moles per g of tissue per 90 min}$, corresponding to 2.7 $\mu\text{moles per g of tissue per min}$. This value is also in good agreement with previous values based on total fructose disappearance at 30 in adult rat liver (68) fructose disappearance in liver homogenates at 37 (57) and conversion to glucose in liver slices at 37 (7). These values are strikingly close to the assay value of between 2.1 and 2.5 $\mu\text{moles per g of tissue per min}$ for fructokinase measured at 25 (3).

The same conclusion applies to aldolase activities, which are somewhat higher than those of fructokinase. Although triokinase activity was the lowest of all three enzymes, the rate of 1 μ mole per min per g of tissue at 25 again appears to be sufficient to account for the known rates of conversion of fructose to glucose.

ENZYME LEVELS IN HEPATIC TUMORS

Several biochemical studies of a series of slow-growing well-differentiated rat liver tumors, induced by feeding chemical carcinogens, have revealed that the neoplastic transformation may occur without the obligatory loss of enzyme associated with normal hepatic function. However no clear pattern of retention or deletion has emerged. Some enzymes are retained, some are lost, some are retained but regulatory control may be reduced or deleted, and in some instances typical hepatic enzymes appear to be replaced by non hepatic or fetal types (for example 41 65 73)

An especially striking feature revealed in earlier studies of rapidly growing liver tumors is the loss of certain enzymes characteristic of hepatic carbohydrate metabolism, such as fructose-1,6-diphosphatase (71) and glucose-6-phosphatase (72). As a result of such metabolic lesions it was proposed that the metabolic pattern of these hepatomas approaches that of the muscle with a capacity to produce large amounts of lactate (6, 69). Subsequent reports of related changes in hepatic carbohydrate metabolism include replacement of glucokinase the predominant glucose ATP phosphotransferases of normal liver by hexokinase(s) in poorly differentiated hepatic tumors (57 75) and also an apparent tendency toward increase in the molecular species characteristic of non hepatic or fetal liver aldolase with increasing growth rate of liver tumors (49 55 64).

As discussed earlier there is a clear-cut difference between the metabolic utilization of fructose in liver and in muscle (27). In the liver fructose is metabolized by fructokinase and aldolase and the molecule enters into glycolysis or glycogenesis at the triose phosphate stage. In muscle fructose is phosphorylated by hexokinase and it undergoes subsequent reactions as F-6-P. As a result of this difference, and because several properties of carbohydrate metabolism in some liver tumors tend to approach the metabolic pattern of muscle, it became of interest to assay the behavior of fructose metabolizing enzymes in liver tumors of varying growth rate.

With only a few exceptions, all three enzymes of fructose metabolism were present in well-differentiated liver tumors (2 17). Fructokinase and triokinase were much lower in activity than in liver but aldolase, though somewhat variable, was usually in the range of liver activity and the low FDP F 1 P activity ratios indicated that the aldolase was nearly exclusively of the liver type. These findings represent only a small portion of a growing body of evidence that many enzymes associated with liver-specific functional activities are retained in these chemically induced, transplantable tumors, not only through the initial neoplastic transformation, but through many transplant generations (41). In contrast, the poorly differentiated tumors were devoid of fructokinase and triokinase, and the aldolase activity though high, was predominantly or entirely of the non-hepatic type. More recently aldolases were purified to homogeneity from rat liver muscle and the Novikoff or Yoshida hepatoma (both characteristic of poorly differentiated liver tumors) (9 20 29). Rigorous characterization in physical and chemical terms provided support for the conclusion that liver aldolase is replaced by muscle aldolase during the development of these liver tumors.

The enzyme data are in good accord with previous results on fructose metabolism.

Sweeney et al. (65) studied the metabolism of fructose- $U^{14}C$ in slices of some of these tumors and found that net fructose uptake was lower in tumors than in liver its conversion to glucose also was very low and was decreased further in tumors with high growth rate. However conversion of the labeled carbon to CO_2 , lactate and glycogen was actually higher in the fast growing, poorly-differentiated tumors. Although at first glance these findings appear to be incompatible with the lack of fructokinase in the poorly-differentiated tumors, these tumors do have high hexokinase activity (58) and fructose, in the concentrations used by these investigators, namely 30 mM, is a good substrate for this enzyme. The low rate of conversion of fructose to glucose can be attributed to low or negligible levels of fructose-1,6-diphosphatase and glucose-6-phosphatase in these tumors (70). Experiments of Ashmore et al. (5) with fructose- ^{14}C provided further evidence for utilization of fructose via fructokinase in well-differentiated tumors and via hexokinase in poorly-differentiated tumors. They demonstrated that glycogen synthesis from fructose in slices of poorly-differentiated liver tumors proceeded without appreciable cleavage of hexose, therefore via hexokinase, whereas in the well-differentiated 7800 and 5123D tumors it occurred via intermediary triose formation, thus presumably via fructokinase.

From previous work on these three enzymes in liver (see above and 61) it appears that they respond to dietary changes as a unit, suggesting that their control may be coordinated. Weber (70) had proposed that hepatic enzymes involved in sequential metabolic steps may be regulated as a single unit. Despite a few glaring exceptions, it is clear that in the well-differentiated tumors all three enzymes are usually present, whereas, in the poorly-differentiated ones all are absent or nearly so. Within this pattern, however there are such wide variations in

activity that one must assume such coordination does not extend to the quantitative expression of enzyme activities.

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ASPECTS OF FRUCTOSE METABOLISM IN NORMAL MAN

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Abstract. In normal subjects infusion of fructose (1 g/kg/hr) for 4 hrs resulted in an increase in the glycogen content of the m. quadriceps femoris of 3.3 g/kg wet muscle (muscle samples obtained by needle biopsy). This was equal to the amount of glycogen formed after glucose infusion of the same magnitude. In muscle depleted of glycogen by exercise, infusion of glucose resulted in twice as much glycogen formed, as did fructose infusion. Formation of liver glycogen was much higher after fructose than after glucose infusion (liver samples obtained by Menghini biopsy). Studies by hepatic vein catheterization indicated that glucose formation by the liver was insufficient to account for the synthesis of muscle glycogen, which presumably occurred directly from fructose taken up by the muscle.

The occurrence of high lactic acid concentrations in blood resulting from infusion of fructose could be partly abolished by simultaneous infusion of amino acids, thus lessening the risk of lactic acidosis.

In 1874, Kùlx (16) first reported that diabetic patients were able to use fructose administered orally better than other sugars. Since this time many studies have been performed with the purpose of establishing how fructose

is utilized and metabolized in man. This is of particular interest as fructose is widely used for parenteral nutrition with carbohydrate.

For this purpose fructose is stated to have several advantages over glucose (25). Fructose is more rapidly utilized than glucose resulting in a lower total blood sugar concentration, when equivalent amounts of either are given (4, 20, 29). It has been reported that postoperative and uremic patients with glucose intolerance can utilize fructose in a normal manner (10, 17). Since fructose is not dependent upon insulin for intracellular penetration, it has been considered to be of value in cases of diabetic acidosis and/or after total pancreatectomy (9, 21). Finally fructose is stated to have a less irritating effect upon the peripheral veins than does glucose, which implies that solutions for intravenous infusion can be given in much higher concentrations (26).

It has been known for almost two decades that fructose administered intravenously is rapidly taken up by the liver and partly metabolized to lactic acid which is subsequently released into the general circulation

(20). The clinical implication of this was pointed out by Bergström et al., in 1967 (5) who demonstrated that this formed lactic acid could give rise to pronounced metabolic acidosis which could be potentially dangerous in patients with a disturbed acid-base balance. This was confirmed by Andersson et al (2) who reported that fatal acidosis could ensue following massive intravenous administration of fructose to children with metabolic acidosis.

In addition to accumulation of lactate, a significant elevation in the plasma levels and the urinary excretion of uric acid was demonstrated in man following rapid administration of fructose (25). This was ascribed to rapid depletion of ATP in the liver resulting from phosphorylation of fructose (19) to fructose-1 P (8, 32).

Oral feeding of fructose or sucrose has been reported to produce hyperlipemia in man, which has been implicated in the etiology of occlusive arteriosclerotic disease (e.g. 23, 33). The fructose portion of sucrose appears to be responsible for the hyperlipemic effect.

Our group at St Erik's Hospital has studied the formation and breakdown of glycogen in human muscle tissue (quadriceps femoris) with the needle biopsy technique (3, 14). More recently similar studies of liver glycogen in normal man have been performed. The Menghini technique for liver biopsy was used. We have also applied the technique of liver vein catheterization with measurement of splanchnic blood flow with BSP or cardio-green, and determination of metabolites in arterial and hepatic venous blood.

In the following are briefly reported some results in connection with fructose administration. Some recent data on the modification of lactate formation by simultaneous intravenous administration of amino acids will also be presented.

Formation of muscle and liver glycogen after glucose and fructose infusions was studied in normal healthy volunteers. Glu-

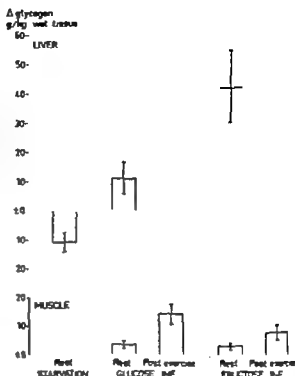


Fig 1 Changes in liver and muscle glycogen during fructose or glucose infusion (1 g/kg b.w./4 hr). For liver glycogen the columns denote changes from values obtained after an overnight fast. A decrease in liver glycogen content was observed during further 4 hours' starvation. Increases in liver glycogen were found when fructose or glucose (1 g/kg b.w./hour) were given during 4 hours. Muscle glycogen was determined before and after an infusion of fructose or glucose at rest or following heavy exercise with one leg.

cose or fructose were administered intravenously as a constant infusion (20 ° solution) 1 g/kg body weight per hour during approximately 4 hours. In the first experiments only muscle biopsies were obtained before and after the infusion (4). In later studies (24) liver biopsies were also obtained before and after administration of fructose (3 subjects) or glucose (7 subjects). These results are summarized in Fig 1. From these it can be seen that in resting human subjects following an overnight fast, there is a decrease in liver glycogen content during a further 4 hours' starvation. If an infusion

of fructose or glucose is given over a corresponding period of time, there is a considerable increase in the liver glycogen content. The increase was found to be much higher with fructose than with glucose, indicating that the former is more efficiently utilized for glycogen formation in the liver than is the latter. This is in agreement with earlier studies made by Cori (7) who demonstrated that fructose was more rapidly metabolized in the rat liver than was glucose.

In muscle, on the other hand, the rate at which glycogen formation occurs following administration of either fructose or glucose, seems to be primarily dependent upon whether the muscle glycogen level is normal or has been lowered by exercise. In resting subjects with a normal muscle glycogen content formation of glycogen, following either fructose or glucose infusion, was on average the same. However in muscle previously depleted of glycogen by heavy exercise (in all these experiments with only one leg) glycogen resynthesis after glucose infusion was about twice as fast as after fructose infusion. This difference was highly significant. The higher rate of glycogen formation following glycogen depletion by heavy exercise may at least be partly explained by the transformation of glycogen synthetase from the D to the I form (the I form being considered to be the active form *in vivo*) (8). The discrepancy between the results obtained with fructose and glucose may be due to a) the higher stimulation of insulin release during the glucose infusion, and to b) the lower total supply of carbohydrate to the muscles by the blood stream during the fructose infusion.

A very interesting problem is whether fructose itself is taken up by the muscle tissue and is used in the synthesis of glycogen or whether it must first be metabolized in the liver or in some other tissue, to glucose or other substrate. Earlier studies in man and experimental animals have yielded conflicting results.

From experiments performed with isolated rat diaphragm it has been shown that fructose is taken up from the medium and assimilated to glycogen. Glycogen formation from fructose, however is much less than from an equivalent amount of glucose. The presence of glucose in the medium decreased the uptake of fructose, whereas an excess of fructose, on the other hand, did not interfere with glucose uptake to any extent (18-22). As glucose is always present in the intact organism it has, thus, been assumed that under physiological conditions fructose is not phosphorylated (13). Contrary to this, studies of peripheral arteriovenous difference of fructose, during infusion of fructose, have indicated that in both man and dog there is an actual uptake of fructose presumably by the skeletal muscle tissue (15, 30-31).

We approached this problem indirectly by measuring the output of glucose from the splanchnic area during a fructose infusion. One of these experiments is illustrated in Fig 2. In this it can be seen that a fructose infusion caused a decrease in the production of glucose from the splanchnic area, a negative *v-a* difference being recorded during part of the infusion. As this occurred in spite of a moderate increase in blood glucose concentration, it indicates that peripheral glucose consumption diminished more rapidly than glucose production from the liver. Similar results have been obtained in other experiments (4), cf also Fig 4.

In the experiment illustrated in Fig 2 it can also clearly be seen that the main source of lactate during fructose infusion is the liver which rapidly removes fructose from the arterial blood.

One explanation of these results may be that lactate formed in the liver is taken up by the muscle and metabolized to glycogen by gluconeogenesis. This, however is unlikely since it is known that the gluconeogenic pathway is absent or of low capacity in skeletal muscle. Furthermore the amount of lactate formed during the infusion period

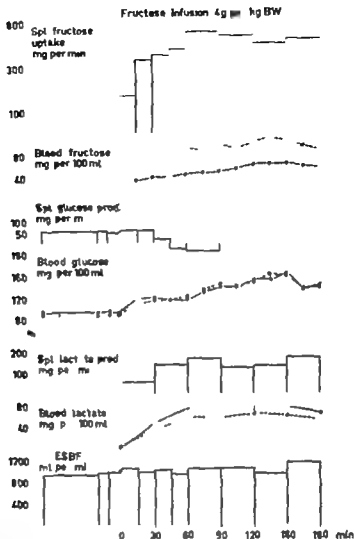


Fig. 2. Estimated splanchnic blood flow (ESBF) and splanchnic metabolism before and during infusion of 30 g fructose to a normal subject.

— arterial blood

— liver vein blood

(25 g) was insufficient to account for the total increase in muscle glycogen (80 g assuming a muscle mass of 20 kg) especially when considering that about 20 g were present in the body fluids (assuming an even distribution in a total body water volume of 35 kg).

In the experiment illustrated in Fig. 2 a catheter was also placed in the renal vein and the renal glucose uptake/production was calculated from the renal blood flow (deter-

mined by the PAH-method) and the $v_a - v_v$ difference for glucose. During the infusion period fructose was taken up by the kidney (and excreted in the urine). During the later part of infusion the renal $v_a - v_v$ difference for glucose was positive, indicating that the kidney was producing glucose. The total production of glucose during the fructose infusion, however, did not amount to more than 6 g a very small amount compared to the total amount of muscle glycogen formed.

LACTATE CONC mmol/L
10-

0 FRUCTOSE FRUCTOSE AMINO ACIDS

6

4

2

0

Fig 3 Blood lactate concentration after infusion of fructose (1 g/kg b.w) or fructose + amino acids (1 g/kg b.w + 50 mg N/kg b.w) during 60 min.

From this and other similar experiments it was concluded that skeletal muscle of man can utilize fructose directly from the blood stream and assimilate it to glycogen. The way in which fructose is metabolized in human skeletal muscle remains obscure. In preliminary experiments we have measured the levels of the hexose phosphates (G-6-P G-1 P F-6-P FDP) and triose phosphates (dihydroxyacetone-P glyceraldehyde-3-P) in skeletal muscle and found only slight increases or no increases in concentration during a fructose infusion. Similar results were obtained when glucose was infused which resulted in pronounced glycogen formation (unpublished observations) Fructose-1 P which is known to increase to very high levels in liver due to phosphorylation of fructose by ketohexokinase was not measured in the muscle samples. However ketohexokinase has not been found to be present in rat skeletal muscle (1).

It has been shown that amino acid solutions given intravenously are better utilized if a carbohydrate is also given simultaneously and that fructose appears superior to

glucose in favouring amino acid utilization (11 12).

In recent experiments we have studied the effect of amino acid administration on fructose metabolism. The solution used consisted of the eight essential amino acids in the mutual proportions recommended by Rose + the non-essential amino acids alanine arginine, glycine serine proline, histidine and aspartic acid, cf Vinnars (27) Table II, group 2. We found that by combining the fructose infusion (1 g/kg/hour) with an infusion of this amino acid solution (0.05 g N/kg/hour) the level of lactate in the blood was much lower than when only fructose was given (Fig 3).

Proceeding from this observation we recently made a few studies with liver vein catheterization during fructose infusion together with amino acids. A typical study performed in a normal man is illustrated in Fig 4. Uptake of fructose over the splanchnic area was high throughout the entire infusion period and did not change appreciably when the amino acids were given simultaneously during the last hour. Splanchnic glucose production fell during fructose infusion as observed in earlier experiments, but increased again when amino acids were administered. The most striking changes were observed in lactic acid production. This, as seen earlier was high during the fructose infusion period, but decreased considerably on addition of the amino acids resulting in a rapid decrease in the blood lactate concentration. This pronounced effect of the amino acids on the production of lactate from fructose by the liver is of considerable interest, since it indicates that fructose can be given with less danger of acidosis from excessive lactate formation, if an excess of amino acids is included in the infusate.

The mechanism(s) through which the above phenomena are brought about remain obscure. One possibility is inhibition of lactate formation through blocking of some

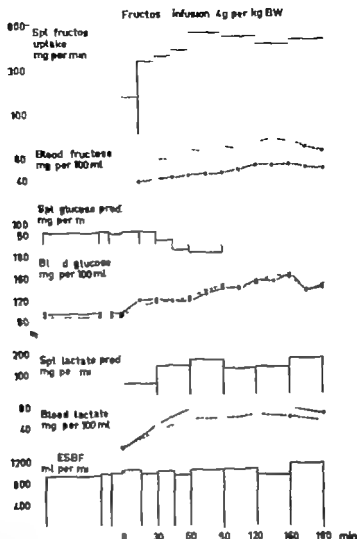


Fig 1 Estimated splanchnic blood flow (ESBF) and splanchnic metabolism before and during infusion of 20 g fructose to a normal subject.

--- arterial blood

— liver vein blood

(25 g) was insufficient to account for the total increase in muscle glycogen (60 g, assuming a muscle mass of 20 kg) especially when considering that about 20 g were present in the body fluids (assuming an even distribution in a total body water volume of 35 kg).

In the experiment illustrated in Fig 2 a catheter was also placed in the renal vein and the renal glucose uptake/production was calculated from the renal blood flow (deter-

mined by the PAH method) and the v-a difference for glucose. During the infusion period fructose was taken up by the kidney (and excreted in the urine). During the later part of infusion the renal v-a difference for glucose was positive indicating that the kidney was producing glucose. The total production of glucose during the fructose infusion, however, did not amount to more than 6 g, a very small amount compared to the total amount of muscle glycogen formed.

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HEREDITARY FRUCTOSE INTOLERANCE

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Abstract. The clinical and metabolic aspects of hereditary fructose intolerance (HFI) are reviewed and new observations on children with HFI are reported. The acute rise in plasma FFA level which was earlier shown to follow an acute exposure to fructose in these patients, was found to be associated with an increase in synovial effusion and a decrease in plasma TG level. Abolition of the fructose induced hypoglycemia by co-administration of glucose after injection of fructose prevented the rise in plasma FFA, but did not modify the increase in plasma lactate or uric concentration. Maintaining normal or high plasma inorganic phosphate levels after fructose ingestion by infusion of phosphate buffer did not alter the fructose-induced changes in blood glucose or lactate, or in plasma FFA or uric concentration. The hyperuricemia and hypoglycemia after fructose were larger in HFI patients than in normal children. Administration of fructose in the small doses of 0.33-1.3 g/kg daily for 3-5 days brought about gradual fall in plasma cholesterol level, and a rise in the excretion of uric acid and 17-ketogenic steroids, but no increase in proteinuria.

Several common dietary constituents are actually poisonous to individuals who are afflicted by a genetic defect or impairment in the metabolism of these compounds. Hereditary fructose intolerance (HFI) is an example of this kind of defect, but unlike many conditions of this type, it is very difficult (e.g. due to the complexity of the harmful substance) to avoid in the diet. In fact, aversion to sweet foods, which usually develops in the affected person and a fructose-free diet become self-enforced. The person may lead an essentially healthy life and be totally unaware of the serious nature of his distaste. The widespread practice of using fructose for intravenous nutrition may risk the life of such an individual in the course of some incidental illness. On the other hand many infants with HFI die of the disease as they are unable to accept breast feeding and as the serious role of fructose in causing the severe illness of an infant is hardly recognized by a nurse or doctor who is not specifically alerted.

Abbreviations: ATP, adenosine triphosphate; F, fructose; FFA, free fatty acids; HFI, hereditary fructose intolerance; KCl, potassium chloride; P, inorganic phosphate; TG, triglyceride.

HFI is apparently not very rare. About one hundred cases have been reported in the literature, many more have been diagnosed in several countries, and most of the patients probably still remain unrecognized. In Finland, we know of 18 patients belonging to 14 families.

DIFFERENT CLINICAL MANIFESTATIONS

The severity of the clinical presentation of HFI depends on the intake of fructose: no symptoms are associated with the metabolic anomaly in the absence of dietary fructose. Young infants tend to have a chronic serious disease, usually dominated by signs of liver damage. After the first year of life, periodic vomiting may occur which will probably be labelled as acetonemic (7). Accidental deviations from the self imposed dietary restrictions may cause attacks of abdominal pain, nausea, vomiting and hypoglycemic symptoms. In adults renal calculi (23), polyuria and periodic or progressive weakness or paralysis (31) have been reported as results of chronic renal tubular dysfunction and hypokalemia. An enlarged liver is common to all forms of presentation in infancy and childhood, but may not be notable in adults (18).

The infantile symptomatology deserves some elaboration. Poor appetite and vomiting with resulting slow gain or even loss of weight and hypochromic anemia are the most regular features. The vomiting varies from projectile to spitting. Thus, the clinical picture varies from one of failure to thrive to one of pyloric stenosis. Periodic symptoms of hypoglycemia may add to the picture frequently as apneic spells. Icterus is common, especially prolongation of neonatal jaundice, and more serious signs of liver damage may develop: gross hepatomegaly, hypoalbuminemia and abdominal distension with ascites. The spleen is frequently enlarged. Deficiency of hepatic coagulation

factors is also common, and hemorrhage tendency may dominate the picture with large gastrointestinal or cerebral bleeding (49). The urine usually contains protein and increased amounts of amino acids. Metabolic acidosis may be severe: it is partly of renal tubular origin but hyperlactatemia (9, 44) is a contributing factor. If plasma amino acids are analyzed, large elevations in the concentrations of tyrosine and methionine may be found and the condition may be called acute tyrosinosis (20, 28, 54). Infantile HFI may thus appear under a variety of diagnostic labels, partly depending upon the availability of laboratory facilities.

Some infants may be able at the age of a few months to convey their distaste to sweet tasting foods to an intelligent mother and remain healthy even after weaning from breast. Any infant with an enlarged liver and protein- or aminoaciduria is so suspected having HFI that fructose should be excluded from the diet, until the diagnosis has been ruled out. Similarly parenteral fructose ought not be given to any patient not positively known to tolerate sugar.

ENZYME DEFECT AND SECONDARY METABOLIC DISTURBANCES

The basic defect in HFI is a gross deficiency in the activity of aldolase B, also called liver aldolase (Fig. 1) (21, 32, 35, 38, 39, 52) which is normally present in the liver, renal cortex and intestinal mucosa. This enzyme provides practically all of the fructose-1-phosphate splitting activity and also most of the fructose-1, 6-diphosphate splitting activity of these organs. The rest of the latter activity is due to a different molecule, aldolase A or muscle aldolase (42). The mutation probably involves the structural gene for the B aldolase. This is suggested by the demonstration in some patients of material that crossreacts with antibody to normal B aldolase but has little measurable enzyme activity (40).

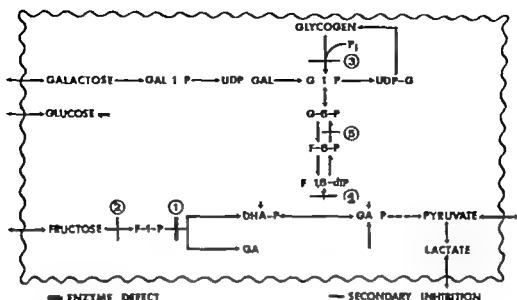


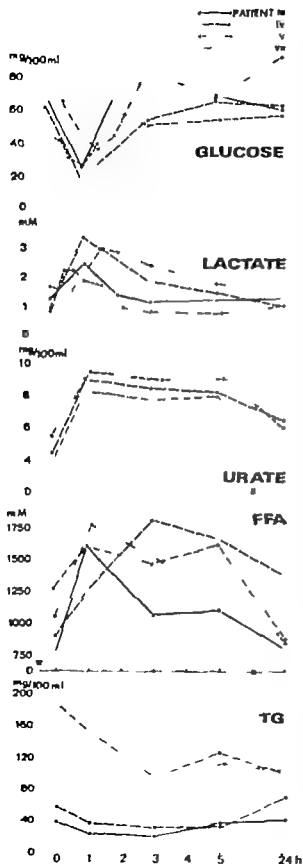
Fig. 1 Metabolism of fructose in the liver cell. The enzyme deficient in hereditary fructose intolerance is indicated by 1. Secondary inhibition of metabolic steps by accumulating F 1-P is indicated by 2 (fruct kinase), 3 (phosphorylation of glycogen) 4 (synthesis of F 1, 6-diP by

aldolase), and 5 (conversion of F-6-P to G-6-P by phosphoglucose isomerase).

Abbreviations F fructose, G glucose, Gal, galactose, P phosphate, P inorganic, P UDP uridine diphosphate, DHA, dihydroxyacetone, GA, glyceraldehyde.

The immediate metabolic disturbance caused by fructose in absence of adequate F 1-P aldolase activity is accumulation of the substrate F 1-P in the three organs, as they also possess fructokinase and are thus able to phosphorylate fructose rapidly. Interestingly F 1-P probably accumulates briefly even in the normal organism after a fructose load (4, 56), and many of the metabolic consequences of fructose in HFI can be regarded as marked exaggerations of events taking place in normal individuals. Some of these metabolic consequences are effects of the accumulating F 1-P some are due to sequestration of inorganic phosphate as F 1-P and resulting intracellular deficiency of P_i. Temporary depletion of ATP is probably a further factor. The acute functional derangements in organs other than the primary three are probably mediated by hypoglycemia (Fig. 2) or acidosis.

Increased utilization of glucose due to increased insulin activity is not responsible for the fructose-induced hypoglycemia in HFI. This is shown by the decrease of plasma insulin levels (9, 18) and by the increase of plasma FFA concentration (9, 37) after fructose administration. The mechanism of the marked hypoglycemic response, which after large doses may result in zero blood glucose level, is inhibition of hepatic output of glucose. This has been shown by injecting a tracer dose of radioactive glucose and demonstrating a decrease in the rate of fall of blood glucose specific radioactivity when fructose is injected (14). This indicates that the production of glucose is inhibited both from liver glycogen (as shown also by the lack of glycogen response during the fructose effect (9, 18, 44)) and from gluconeogenic sources. An inhibition of glucose-6-phosphatase would explain both, but such a block obviously is not



present since galactose is effectively converted to glucose and is able to relieve fructose-induced hypoglycemia (9, 19, 50). In fact, this finding indicates that the inhibition of glycogen breakdown must take place at or above the phosphorylase reaction. Depletion of the liver cell of P_i , which is needed in the phosphorylase, is one possible mechanism, particularly as the sequestration of phosphate is manifested as a fall in plasma phosphate concentration even before the hypoglycemic response sets in. However the fructose-induced hypoglycemia is not influenced by phosphate infusion which prevents the depletion of P_i (Fig. 4 ref. 11, 12).

Two factors are known which probably contribute to the inhibition of hepatic gluconeogenesis. Aldolase A, which provides practically all the fructose-1,6-diphosphate splitting and building activity in the absence of active aldolase B in HFI is inhibited by the accumulating F 1 P (16) (Fig. 1). The conversion of fructose-6-P to glucose-6-P by phosphohexose isomerase has also been reported to be inhibited by F 1 P (57). Depletion of ATP may also be involved.

Blood lactate concentration rises after fructose load in HFI patients as sharply as in normal subjects (Fig. 2, Table 1). In normal subject lactate is thought to be derived directly from fructose, which enters the glycolytic pathway more rapidly and to a greater extent than glucose. The immediate source of the excess lactate and the mechanism of its generation in HFI are unknown, but it seems unlikely that the metabolic path-

Fig. 3. Blood glucose and lactate, plasma urate, unesterified fatty acid (FFA) and triglyceride (TG) concentrations in 4 children with hereditary fructose intolerance following an oral fructose load. After an overnight fast, 0.4 g/kg (0.7 g/kg in case III) of fructose was ingested and food was withheld for the next 5 hours. The last blood sample was drawn after a overnight fast. Glucose, lactate and urate were determined with specific enzymatic methods. FFA and TG according to ref. 83 and 6.

way from fructose to lactate is similar in normal and HFI subjects. Neither is the lactate production dependent on the hypoglycemia, since fructose injection is followed by hyperlactatemia also if normoglycemia is maintained by glucose infusion (Fig 4).

The plasma FFA concentration rises sharply in patients with HFI after the administration of fructose (Fig 2, ref. 37). This rise seems to be dependent on the hypoglycemia as it can be inhibited by glucose infusion (Fig 4). It is probably evoked by increased lipolysis of adipose tissue triglycerides resulting from hypoglycemia induced release of epinephrine (Fig 3), corticotropin (as reflected by an increase in the excretion of 17 KGS, Fig 3) and growth hormone (9, 23). Another factor which might be thought to contribute to the rise of plasma FFA is decreased re-esterification in adipose tissue caused by hypoglycemia and concomitant decrease of α -glycerophosphate (37). This mechanism is less plausible, however since fructose is readily metabolized in adipose tissue (15) also in HFI and provides α -glycerophosphate. The production of ketone bodies is enhanced by administration of fructose in HFI (7, 44) in analogy with other situations associated with increased mobilization of FFA.

Normally the liver extracts FFA in pro-

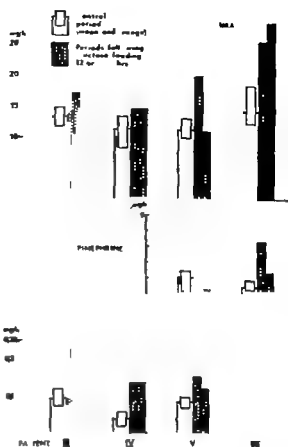
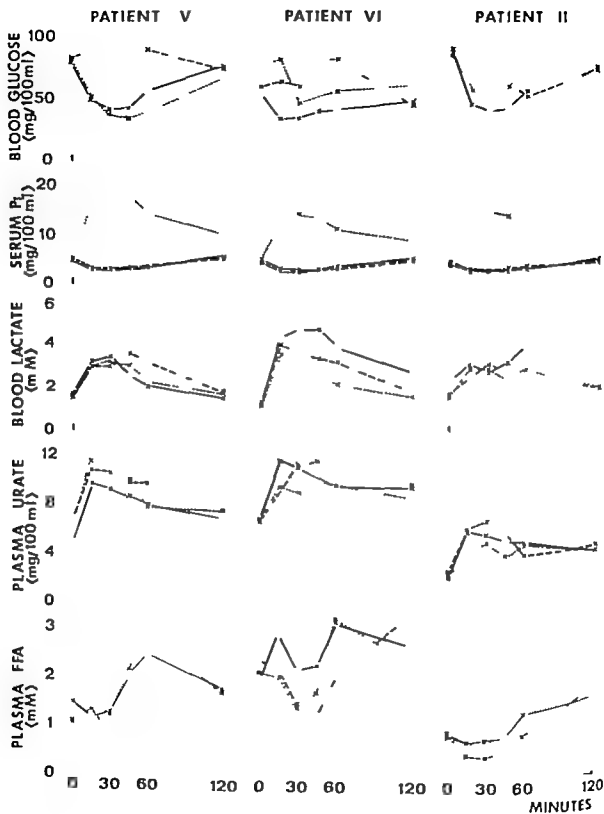


Fig 2. Urinary excretion of urate, epinephrine (16) and 17 ketogenic steroids (17 KGS) (3) in 4 children with hereditary fructose intolerance during control periods and after fructose administration as described in Fig. 2.

Table I. Maximal changes measured in blood glucose and lactate, and in plasma urate concentration after fructose in 5 normal children and in 5 patients with hereditary fructose intolerance

	Normal children		HFI patients	
	Mean	Range	Mean	Range
Urate mg/100 ml	+ 2.8	1.9—4.5	+ 4.5	3.5—5.0
per cent	+ 82	85—168	+ 113	77—181
Lactate mg/100 ml	+22.6	21.3—24.5	+21.5	10.9—30.4
per cent	+ 149	112—175	+ 188	74—287
Glucose mg/100 ml	+ 55	13—82	— 39	—20—49
per cent	+ 88	20—96	— 53	—36—72

The dose was 0.6 g/kg in the normal children and 0.4 g/kg in the HFI patients, given as a 2 min intravenous injection. All measurements were done with specific enzymatic methods.



portion to their concentration in plasma and rapidly esterifies them to triglycerides and phospholipids, which are released into the circulation in the form of lipoproteins. Increased plasma FFA concentration induced by epinephrine injection is associated with a rise in plasma TG concentration in normal subjects (for a review see 36). In contrast, the rise of plasma FFA after fructose in HFI is associated with a fall in plasma TG concentration (Fig 2). This may be due to impairment of the hepatic uptake or esterification of FFA, or due to an inhibition of release of TG or a combination of these factors. Since both glucose and insulin are known to stimulate plasma TG production it is possible that hypoglycemia and low plasma insulin level are responsible for the acute decrease of plasma TG after fructose. The fact that heavy accumulation of fat in the liver is a very characteristic histologic finding in HFI patients exposed to fructose (24, 41-44) suggests that the release of TG from the liver is inhibited. In contrast to TG which in the fasting state originates solely from the liver the plasma cholesterol concentration showed no consistent alteration during the acute exposures to fructose. During prolonged exposures to small amounts of fructose, the plasma cholesterol level fell gradually in all patients studied (Table 2), whereas fasting plasma TG and FFA showed

no change. The lowering of the cholesterol concentration could be due to a direct effect of fructose on the liver and/or intestinal mucosa.

The observation of a heavy urate sediment in the urine of HFI patients during and after exposure to fructose led us to the discovery that urate production is increased by fructose both in HFI patients and in normal subjects (45). The mechanism behind this effect was subsequently shown to be an increased breakdown of liver adenine nucleotides (30-47), apparently induced by the depletion of P in the liver cell. As the accumulation of F1P and hence the sequestration of P are thought to be more marked and more prolonged in subjects with HFI than in normal individuals, one would expect the hyperuricemic effect of fructose to be more marked in HFI, too. Our data (Table I) and those of others (34) indicate that this difference is present even though it is not large. This is further supported by the observation that a prolonged exposure to fructose causes a significant increase in urinary urate excretion in HFI patients (Table II), but not in normal subjects. The fructose-induced hyperuricemia in HFI patients is not prevented by phosphate or glucose infusions (Fig 4).

Vomiting and nausea are characteristically experienced by HFI patients after oral intake of fructose. It is likely that these symptoms are associated with the enzyme defect and F1P accumulation in the intestinal mucosa. Fructose seems to impair the absorption of glucose in affected subjects (19-51). There are also some observations suggesting that diarrhea is a component of the HFI syndrome (5-18-25), though inconsistent.

Morris (33-34) has shown that acute loads of fructose impair the reabsorption of bicarbonate, phosphate, amino acids and urate in the proximal tubule. The condition is similar to the tubular disturbance known as the Fanconi syndrome, associated e.g. with cys-

Fig 4. Blood glucose and lactate, plasma inorganic phosphate (P), urate and unesterified fatty acid (FFA) concentrations in 3 patients with hereditary fructose intolerance following an i.v. load of fructose alone (—) fructose with glucose (x x) and fructose with phosphate (x x). 0.4 g/kg of fructose was injected in 2 min as 20 / solution after an overnight fast. On another day the injection was followed by 60 min infusion of 10 / glucose, 0.14 g per kg and h. On yet another day the fructose injection was followed by a 45 min infusion of 0.15 M Na-phosphate buffer corresponding to 15 mmoles of P per kg and 45 min.

Table II. Effect of prolonged exposure to fructose on the plasma cholesterol concentration and the urinary excretion of urate and 17 ketogenic steroids in three children with hereditary fructose intolerance

	Case III	Case IV	Case VII
Daily dose of fructose (g/kg)	0.65-1.30	0.43-0.60	0.33-0.45
Duration of administration (days)	3	3	8
Plasma cholesterol (mg/100 ml)			
Before exposure	262	203	279
Last day of exposure	156	168	200
Urate in 24-h urine (g per g creatinine mean and range)			
During exposure	3.22 (2.02-3.34)	1.25 (1.12-1.48)	0.72 (0.60-1.00)
Control periods (3-5 days)	1.24 (1.04-1.49)	0.94 (0.73-1.10)	0.51 (0.46-0.60)
17-OHCS in 24-h urine (mg per g creatinine mean and range)			
During exposure	26.7 (26.0-31.5)	21.6 (19.7-25.3)	8.7 (8.0-19.9)
Control periods (3-5 days)	17.4 (12.5-22.5)	17.3 (11.7-23.4)	7.8 (7.5-8.6)

The daily dose of fructose was divided into 3-5 portions and given by mouth. Urine was collected in 24-h periods. Fasting blood samples were drawn every morning and analyzed for cholesterol, which in every case showed a gradual decline to the final value shown in the table. There was no consistent change in the FFA or TG concentration. Urinary protein content was also measured: no rise was observed.

tinosis. It may cause renal calculi and potassium depletion. Proteinuria is a rather constant feature of HFI patients exposed to fructose. It has been reported to appear after one 50 g dose of fructose in an adult patient (46). In the experiments shown in Table 2 a prolonged exposure to fructose caused no rise in urinary protein.

GENETICS

In most of the families reported, the occurrence of HFI conforms to autosomal recessive inheritance. A few families are known (16, 29, 55) with an apparent autosomal dominant inheritance. These may be instances of pseudodominance, i.e. marriages between a homozygote and a heterozygote, but real genetic and biochemical heterogeneity of the syndrome is also possible. A test to reveal the heterozygous state for HFI would be of great value. We have measured liver F1P aldolase activities in 5 parents of HFI patients, but the results were not different from controls (46). Beyreus et al. have reported

a higher blood fructose concentration plates during constant fructose infusion in the parents of his HFI patient than in controls (2). This suggests decreased metabolic clearance of fructose in the heterozygous state, but the finding has not been confirmed in a larger series of heterozygotes.

DIAGNOSIS

Any infant presenting any of the clinical features of HFI, discussed above, should be placed on a fructose-free diet. A clear improvement of the condition in a few days suggests HFI. In older patients, the dietary history of aversion to sugar-sugar-containing foods, fruits and berries is almost diagnostic. The diagnosis is confirmed by an intravenous fructose test. 0.25 g/kg is given as a 10% solution in 2 min and blood glucose and serum inorganic phosphate are measured at 20 min intervals for 80 min. A distinct fall occurs in both in HFI. Recently a patient was reported with a defect of liver fructose diphosphatase activity (1). That patient was

indistinguishable from HFI patients on fructose test, but also had fasting hypoglycemia which is not a feature of HFI, and had no aversion to sweet. Two sisters reported by Dormandy and Porter (13) had severe hypoglycemia after both fructose and galactose, but also they had no distaste for sweet.

Another case is on the record (8) with a hypoglycemic response to fructose but with normal activities of F 1 P aldolase and fructose diphosphatase in the liver

TREATMENT

Many of the patients seem able to spontaneously select a fructose-free diet. However strict instructions are necessary (43). Recently glucose was recommended to be added to the diet, on the speculation that the liver fibrosis seen in the patients still after years on a fructose-free diet be due to lack of glucose (27). We are strongly against any trial to break the unique self protection that the patients have developed in their intense aversion to sweet taste, and prefer adding more starch to the diet if the theory turns out to be valid.

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ADDENDUM

After the manuscript was finished studies have been performed in two mothers of HFI patients according to the protocol of Beyreus et al (2). In contrast to their finding no difference from controls was observed (Perheentupa, J and Granstrom, M L, unpublished results)

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HEREDITARY ALTERATIONS OF FRUCTOSE METABOLIZING ENZYMES

Studies on Essential Fructosuria and on Hereditary Fructose Intolerance

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Abstract. Two hereditary alterations of fructose metabolizing enzymes are known. W have shown that in essential fructosuria, there is in fact deficiency of fructokinase activity W have shown that in hereditary fructose intolerance (HFI) some abnormal properties of aldolase in liver are related to aldolases A (muscle type) and C (brain type) which are normally synthesized by embryo, and which persist without change. In livers with HFI, we have found protein immunologically related to aldolase B (liver adult type), the enzymatic activity of which is about 3 per cent of the normal value. Its Michaelis constant for fructose-1 phosphate is greatly increased.

W conclude that, in hereditary fructose intolerance, there is mutation of the structural gene.

As shown by Hers (9) and then confirmed by Sillero et al. (25) the three enzymes of fructose metabolism in liver are fructokinase, liver type aldolase (aldolase B) and triosekinase. Heinz has reminded us of the

normal metabolic pathway. Two metabolic diseases, caused by a deficiency of enzymes of this metabolism are known (5) essential fructosuria (deficiency in fructokinase) and fructose intolerance (deficiency in aldolase B). We would like to study these two enzyme deficiencies here.

FRUCTOKINASE AND ESSENTIAL FRUCTOSURIA

Essential fructosuria was distinguished from hereditary fructose intolerance by Chambers et al. (1) and Froesch et al. (6). These last authors have put forward the hypothesis that the primary enzymatic defect is a lack of fructokinase. We have had the opportunity to confirm this hypothesis (24) and we shall recall briefly our findings, without describing the biological symptoms, or the genetic story: the diagnosis was made by a systematic investigation.

We used the method of Hers and Joassin (10) measuring the amount of fructose-¹⁴C used up by fructokinase. Homogenates were incubated with fructose-¹⁴C, ATP Mg⁺⁺ and

Table I. Fructokinase and aldolase activities in patients with essential fructosuria and with hereditary fructose intolerance

	Activities in μM of substrate utilized per minute and per gram			
	Fructokinase	Aldolase		
		FDP	F 1 P	FDP/F 1-P
Normal adult liver	0.05 to 0.12	6.0 to 14	6.0 to 14	1.0 to 1.1
Hereditary Fructose Intolerance (mean of biopsies, children and infants)	0.06	2.0	0.38	5.3
Essential Fructosuria	0	11.8	11.8	1.0

K^+ and after precipitation with barium, radioactivity of supernatant was estimated in reaction, and in blank without ATP

Under these conditions, normal adult liver used between 0.05 and 0.12 μM fructose per minute and per gram of wet liver. In five livers from patients with fructose intolerance, we found the same values. On the other hand, in the liver of a subject with biological symptoms of essential fructosuria, the two aldolase activities were normal, and we found no fructokinase activity as seen in Table I.

The rarity of this anomaly (which is not a true disease) has not allowed us to perform other investigations on the enzymatic defect.

ALDOLASE AND HEREDITARY FRUCTOSE INTOLERANCE (HFI)

After the lecture of Perbeentupa, we shall confine this chapter to some new data that we were able to bring up on the enzymic defect in HFI.

We confirmed, as other authors, the findings of Hers and Joassin (10). In the liver biopsies of 26 children with HFI we found a mean fructose-1,6-diphosphate (FDP) aldolase activity of 2.0 and fructose-1-phosphate (F 1 P) aldolase activity of 0.38. We point out that the colorimetric method that we used is more exact for the determination of F 1 P aldolase activity than the optical test

(22). The mean aldolase activity ratio FDP/F 1 P was 5.6 while it is normally between 1.0 and 1.1. As pointed out by Hers and ourselves, this high ratio is the characteristic enzymic anomaly in HFI. It is never encountered in other diseases, except in hepatoma, as we have shown (13, 20). We have shown earlier that several properties of embryonic liver were similar to properties of liver with HFI (19). We recall first that three types of aldolase are known (9, 15, 18): aldolase A (muscle type), aldolase B (liver type) and aldolase C (brain type). Table II summarizes the main properties of the three types in human tissues.

Spolter et al. (26) have shown that ATP competitively inhibits muscle-type aldolase but not the liver type. We have found that

Table II. Isoenzymes of aldolase in human tissues

	Types		
	A	B	C
Activity ratio FDP/F 1 P	> 50	1.0	5 to 10
Electrophoretic migration (in starch gel) at pH 7.0	Anodic (slow)	Anodic (slow)	Anodic (fast)
Tissues (in which this type is preponderant)	Muscle	Liver	Brain

Table III. Inhibition of FDP activity

	Inhibition of FDP activity		
	Aldolase activity ratio FDP/F 1 P	Percentage of inhibition by ATP	Percentage of inhibition by antiserum antialdolase A
Human muscle	38	45	95
Human adult liver	10 to 11	< 18	< 18
Human fetal liver (2nd to 4th month)	2.0 to 3.0	25 to 35	30 to 45
Liver with HFI	5.6 (mean of 26 biopsies)	30 (3 experiments)	40 to 60

Concentration of ATP: 15 mM

Concentration of FDP: 0.06 mM

Antiserum antialdolase A is prepared in chicken by repeated injections of crystalline rabbit muscle aldolase. This antiserum, like the antiserum antialdolase B, exhibits a good type specificity but a poor species specificity.

aldolase of fetal liver and of liver with HFI is inhibited by ATP and also by the specific antiserum antialdolase A (muscle type). Table III summarizes our findings.

We see that liver with HFI, like fetal liver contains muscle type aldolase. More recently our team has shown by kinetic, electrophoretic, and immunological methods, that fetal liver also contains brain type aldolase (aldolase C) (21, 22). We are now able to characterize with electrophoresis, according to the method of Penhoet et al (15) the FDP aldolase activity in three livers with HFI. As shown in Fig 1, the pattern is similar to the brain pattern, and it seems that in HFI liver there are (as in fetal liver) hybrids of the two aldolases A and C.

Are the aldolases A and C really increased in liver with HFI? It seems to us that these embryonic forms are without change and only relatively increased. We recall that the specific activities of aldolases A and C are much greater than that of aldolase B: an amount of 2 or 3 per cent may represent about 15 per cent of total FDP activity. We

have consequently proposed that the abnormal properties of aldolase in HFI are related to aldolase which is normally synthesized by

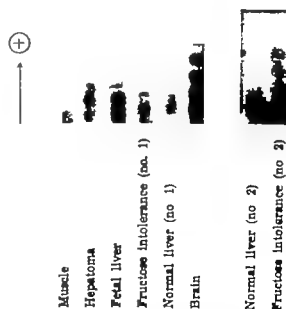


Fig 1. Aldolase isozymes of human tissues (substrate FDP). The concentration of liver HFI extract is much greater than the concentration of the other extracts.

Table IV Percentage of protein cross reacting (CRM) with aldolase B in livers with fructose intolerance

Human tissues	Proteins mg/0.1 ml	Removal of neutralizing antibody (units) (%)	Normal liver proteins mg/0.1 ml (%)	CRM percentage (%)
Fructose Intolerance				
Liver a	0.75	0.66	0.18	24
b	0.80	0.71	0.24	30
c	1.16	0.34	0.21	18

(a) One unit Amount of antibody inhibiting 0.06 unit of FDP cleavage activity

(***) Amount of normal liver proteins producing an equivalent removal of antibody

(***) $\frac{\text{column 3}}{\text{column 1}} \times 100$

embryo and which persists without change. Aldolase B activity would be almost entirely lacking so that the aldolase A (and also the aldolase C) would become the main aldolase present in these livers.

But is aldolase B synthesized in livers with HFI? We brought evidence for the presence of a protein sharing the immunological properties of aldolase B in the liver of three patients (14-23).

Antiserum antialdolase B was prepared in chicken by repeated injections of pure aldolase B (according to the method of Morse and Horecker (12)). We have compared the removal of neutralizing antibodies by normal liver and by liver with HFI. After incubation, and centrifugation, the supernatant was assayed for residual antibody by determining the inhibition of FDP activity of pure aldolase B.

The amount of Cross Reacting Material (CRM) in the studied tissue was estimated by plotting our results on a standard curve where increasing amounts of liver proteins were incubated with 0.1 ml of antiserum anti aldolase B.

Under these conditions, we have found in three livers respectively 4, 18 and 30 per cent of the normal content of an immunological aldolase protein, as shown in Table IV

Comparing this amount of immunologically reactive protein and the F 1 P aldolase activity (which is about 2 to 3 per cent of the normal) we concluded that a Cross Reactive Material (CRM) does exist in these three patients.

Consequently we may propose that the synthesis of aldolase B persists, but that the enzyme is abnormal and almost without catalytic activity. By the method of double immunodiffusion of Ouchterlony we were able to find a band of precipitation between the well with antiserum antialdolase B and the extracts of four different liver biopsies with HFI but these lines exhibited only a partial identity with the precipitation lines between antiserum anti B and extracts of normal liver.

In order to confirm the anomaly of the hepatic aldolase we have estimated the Michaelis constant for its specific (or almost specific) substrate, fructose-1 phosphate because it must be recalled, indeed, that the main part of FDP activity is probably due to aldolases A and C.

Although the F 1 P aldolase activity is very low we have nevertheless succeeded in measuring the affinity of aldolase towards this substrate.



Fig. 2. K_m for the substrate fructose-1 phosphate tolerance. Abscissa $(1/S)$ reciprocal of F 1 P percentage of aldolase activity

in normal livers and livers with fructose intolerance. Ordinate $(1/V)$ reciprocal of per

Fig. 2 shows the results which we obtained in normal adult liver in fetal liver (2–4 months) and in seven livers with HFI

It is seen that the K_m for F 1 P is between 2 mM and 3 mM in normal livers (adult or fetal) Görtler et al. (8) found the same value after purification. On the contrary we find for these pathological livers, values from 10 to 60 mM. In two more recent biopsies we have found a K_m of about 30 mM.

Moreover we have recently found that if our extraction is performed with a buffer with β -mercaptoethanol and EDTA the affinity towards F 1 P becomes normal. On four biopsies where we measured the K_m , either in a water extract or in a β -ME buffer extract, we have found respectively a K_m between 15 and 20 mM in water and less than 3 mM in β -ME buffer with EDTA.

On the contrary normal liver and brain (which exhibits a weak activity for F 1 P) show the same affinity for F 1 P whether they are extracted by water or by the β -ME buffer respectively 2 to 3 mM for liver and about 10 mM for brain.

We have found an abnormal affinity for

F 1 P in the 10 biopsies which we were able to study. On the other hand, we found a CRM only in three biopsies. In the three others, less than 10 per cent of CRM may be found. We have no explanation yet for this discrepancy. It cannot be stated whether the protein is abnormally unstable or whether there is a true lack of synthesis of this protein in some cases.

In the latter case, we should have to conclude that there is a genetic polymorphism in cases with CRM, the mutation would be on the structural gene in other cases on a regulation gene. We recall the findings of Goedde and Altland (7) on the deficiency in serum pseudocholinesterase, where the percentage of protein immunologically related to the normal enzyme varied from 0 to 90. We recall also the analogous findings on the deficiencies in catalase (4) and in hypoxanthine guanine phosphoribosyl transferase (11). Nevertheless, our findings on the abnormal affinity in all cases are in favour of a structural mutation.

We should also like to discuss briefly the genetical problem. Raivio et al. (16) have studied liver biopsies from parents of patients with HFI, and have found an aldolase activity ratio close to the normal one. Nevertheless, we do not think that these interesting

We have verified by testing the action of antiserum anti-B that the F 1 P activity of livers with HFI is really due to aldolase B and not to aldolase C.

Table V Calculation of aldolase activities in heterozygote (percentage of normal activity)

	Aldolase activities		
	FDP	F I P	Ratio FDP/F I P
ALDOLASE B			
— synthesized by the normal gene	50	50	
— synthesized by the mutated gene	1.5	1.5	
ALDOLASES A and C	10	0.5	
Total activity	61.5	51	1.2

results bring evidence for a mutation on a regulation gene with normal aldolase B in the heterozygote. In fact, the calculation shows that in heterozygote with a normal and an abnormal gene for aldolase B, the aldolase activity ratio could be no higher than about 1.2 (Table V).

But the problem of normal FDP aldolase activity in parents of these patients as found by the authors is not solved.

CONCLUSIONS

It seems that in HFI there is a mutation of the structural gene. Recently several examples of genetic diseases with synthesis of an abnormal protein without, or almost without, enzymatic activity have been described (1-3, 7). Dreyfus has emphasized arguments in favour of structural mutations, and against mutations on a regulation gene in inherited diseases (4). We believe that this concept is valid for HFI, and we hope that subsequent work will make it possible to determine more precisely the structural anomaly of aldolase in HFI, and also perhaps of fructokinase in essential fructosuria.

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**METABOLIC EFFECTS
OF FRUCTOSE**

HEPATIC ACCUMULATION OF METABOLITES AFTER FRUCTOSE LOADING

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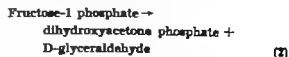
Abstract. The changes in the metabolite content in freeze-clamped livers of fed rats occurring on perfusion with 10 mM D-fructose have been examined under aerobic and anaerobic conditions. During aerobic perfusion the main effects of fructose were an accumulation of fructose 1-phosphate, as already known, up to 8.7 $\mu\text{mol/g}$ of liver within 10 min, a loss of total adenine nucleotides (up to 35 % after 40 min) with decrease in the ATP content to 23 % within 10 min, a seven fold rise in the concentration of IMP to 1.1 $\mu\text{mol/g}$ and an eight fold rise of α -glycerophosphate to 1.1 $\mu\text{mol/g}$. There was transient decrease in P from 4.3 to 1.7 $\mu\text{mol/g}$. Within 40 min the P content recovered to the normal value. The content of lactate increased to 4.3 $\mu\text{mol/g}$ & 80 min pyruvate also increased and the [lactate]/[pyruvate] ratio remained within physiological limits. The concentration of free fructose within the liver remained much below that in the perfusion medium, indicating that the rate of penetration of fructose into the tissue was lower than the rate of utilisation. The fission of fructose 1-phosphate by liver aldolase is inhibited by several phosphorylated intermediates, especially by IMP. This inhibition is competitive with a K_i of 0.1 mM. The maximal rates of the enzyme synthesising and splitting fructose 1-phosphate are about equal. The accumulation of fructose 1-phosphate on fructose loading is due to the inhibition of the fission of fructose 1-phosphate by the IMP arising from the degradation of the adenine nucleotides. When the conditions were anaerobic fructose was readily con-

verted to lactate and the fructose 1-phosphate content of the liver after 40 min rose to 8.8 $\mu\text{mol/g}$. The total adenine nucleotide content decreased to 1.74 $\mu\text{mol/g}$. The contents of glycerophosphate and lactate were 4.3 $\mu\text{mol/g}$ and 5.6 $\mu\text{mol/g}$ respectively the [lactate]/[pyruvate] increased to 83.

D-fructose is metabolised in the mammalian liver mainly by the ketohexokinase (EC 2.7.1.3) reaction to fructose 1-phosphate (19 25)



The further metabolism of fructose 1-phosphate is via the ketose-1-phosphate aldolase (EC 4.1.2.7) reaction.



Assays of these two enzymes in rat liver indicate that the rates of reaction are about the same. Fructose can be metabolised to

fructose 6-phosphate by the hexokinase (EC 4.1.1) reaction but the activity of this enzyme is low in liver (16) and it has a K_m of 2.4–6.7 mM for fructose (45) while that of ketohexokinase is 0.2–0.5 mM (1, 31). In addition, the formation of fructose-6-phosphate via hexokinase is slow in the presence of glucose (47).

In 1942 Kjerulf-Jensen (22) found accumulation of fructose-1 phosphate in mammalian liver after a whole body fructose load (see also ref 9 15). Fructose is also rapidly metabolised by the isolated perfused rat liver under aerobic and anaerobic conditions (46, 47) and this paper is concerned with the accumulation of hepatic metabolites, in addition to F1P after addition of D-fructose to the perfusion medium.

MATERIALS AND METHODS

Animals and diets. Female Wistar rats each weighing 170–215 g obtained from Carworth (Europe) Ltd, Alconbury Hunting, U.K., were used and were fed on a standard small-animal diet (Spillers Mills Ltd, Gainsborough, Lincs, U.K.). Water was provided *ad libitum*.

Liver perfusion. The method of liver perfusion used was that described by Hems et al. (18). The perfusion medium consisted of physiological saline (24) with 2.6% bovine serum albumin fraction (Armour Pharmaceutical Co. Ltd, Eastbourne Sussex, U.K.), and aged human erythrocytes, which were thoroughly washed as described by Woods et al. (46) to lower their initial lactate content. The bovine serum albumin was dialysed as a 10% solution (at 4°C) against three changes of physiological saline gassed with $\text{CO}_2 + \text{O}_2$ (9, 35). In anaerobic experiments where O_2 was to be excluded from the perfusion apparatus and medium the conditions of perfusion were altered as previously described (4), i.e. the washed erythrocytes were omitted from the medium and the perfusions were carried out in the presence of cyanide which was added as an 0.2 M solution of KCN neutralised with HCl to give a final concentration of 1 mM in the medium. The medium was gassed with $\text{CO}_2 + \text{N}_2$ (9, 35).

For other methodological details, see Woods et al. (46) and Woods and Krebs (47).

RESULTS

Content of intermediary metabolites in the freeze-clamped perfused rat liver after fructose loading

The main changes in the contents of liver metabolites after addition of 10 mM fructose to the perfusion medium were the accumulation of fructose-1 phosphate and a loss of hepatic adenine nucleotides as described by Mäenpää et al. (27) and Ralvio et al. (33) (Table I).

The concentration of ATP decreased to 23% within 10 min and then slowly increased, reaching 48% of the original value at 25 min. The sum of the adenine nucleotides decreased to 43% in 10 min and was 50% at 80 min. Some of the lost adenine nucleotides appeared as AMP the concentration of which increased seven-fold within 10 min and then decreased, but the lost adenine nucleotides were not fully accounted for by the substances analyzed. This deficit increased with time. The findings of Mäenpää et al. (27) (made under slightly different conditions) suggest that the adenine nucleotides were partly broken down to uric acid and allantoin.

Among the phosphorylated products fructose 1 phosphate increased 40-fold within 10 min to 8.7 $\mu\text{mol/g}$ and α -glycerophosphate rose eight fold to 1.06 $\mu\text{mol/g}$. Dihydroxyacetone phosphate and glyceraldehyde-3-phosphate increased about four fold. The changes in the concentrations of other phosphorylated compounds, though substantial on a percentage basis in the case of fructose 1,6-diphosphate, glucose-6-phosphate, phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate were small in absolute terms.

There was a large decrease in the concentration of P at 10 min. The value of P_i had returned to normal at 40 min and was increased above normal at 80 min. The total phosphorus content of the liver calculated

Table I. Contents of intermediary metabolites in the perfused rat liver

Livers of fed rats were perfused with a medium containing D-fructose (initial concentration 10 mM) and were freeze-clamped at various times after the start of the perfusion. The initial values refer to livers of fed rats freeze-clamped *in vivo* after cervical dislocation. The results are means \pm S.E.M.

Time of perfusion (min)	Livers not perfused		Livers perfused with D-fructose			
	0		10	40	80	
Number of observations	4		4	4	3	
Content of metabolites ($\mu\text{mol/g}$ wet wt.)						
Fructose-1-phosphate	0.23 (2)		8.72 \pm 0.83	7.68 \pm 0.98	2.37 \pm 1.31	
ATP	2.22 \pm 0.07		0.51 \pm 0.16	0.77 \pm 0.11	0.99 \pm 0.01	
ADP	0.78 \pm 0.08		0.66 \pm 0.07	0.20 \pm 0.03	0.50 \pm 0.10	
AMP	0.28 \pm 0.05		0.20 \pm 0.03	0.17 \pm 0.03	0.14 \pm 0.003	
Total adenine nucleotides	3.27 \pm 0.12		1.37 \pm 0.08	1.14 \pm 0.13	1.64 \pm 0.13	
P	4.25 \pm 0.25		1.67 \pm 0.29	2.97 \pm 0.28	5.82 \pm 0.28	
IMP	0.12 \pm 0.03		1.14 \pm 0.06	0.97 \pm 0.13 (3)	2.26 \pm 0.03	
α -Glycerophosphate	0.13 \pm 0.10		1.06 \pm 0.33	0.60 \pm 0.06	0.45 \pm 0.07	
D-Glyceraldehyde	—		—	0.10 (2)	—	
Free fructose	< 0.01		3.86 \pm 0.51	0.82 \pm 0.20	0.22 \pm 0.11	
Glucose	7.22 \pm 0.77		2.29 \pm 0.65	5.01 \pm 0.45	5.91 \pm 0.16	
Lactate	0.79 \pm 0.09		1.34 \pm 0.15	3.57 \pm 0.34	4.22 \pm 0.68	
Pyruvate	0.06 \pm 0.01		0.18 \pm 0.03	0.35 \pm 0.03	0.26 \pm 0.06	
Fructose-1,6-diphosphate	0.02 \pm 0.003		0.10 \pm 0.02	6.03 \pm 0.01	0.02 \pm 0.003	
Glucose-6-phosphate	0.25 \pm 0.03		0.14 \pm 0.04	0.38 \pm 0.04	0.20 \pm 0.06	
Fructose-6-phosphate	0.06 \pm 0.01		0.06 \pm 0.01	0.11 \pm 0.01	0.07 \pm 0.02	
Glucose-1-phosphate	< 0.01		< 0.01	0.01 \pm 0.001	< 0.01	
Phosphoenolpyruvate	0.16 \pm 0.02		0.05 \pm 0.02	0.19 \pm 0.03	0.22 \pm 0.01	
2-Phosphoglycerate	0.04 \pm 0.01		0.03 \pm 0.01	0.07 \pm 0.01	0.06 \pm 0.003	
3-Phosphoglycerate	0.26 \pm 0.06		0.16 \pm 0.03	0.47 \pm 0.06	0.57 \pm 0.06	
Glyceraldehyde-3-phosphate	0.006 \pm 0.0002		0.03 \pm 0.004	6.006 \pm 0.0003	0.007 (2)	
Dihydroxyacetonephosphate	0.04 \pm 0.003		0.16 \pm 0.04	0.06 \pm 0.003	0.06 (3)	
[Lactate]/[pyruvate]	9.60 \pm 0.98		16.1 \pm 1.23	10.4 \pm 1.24	12.0 \pm 0.73	
[Fructose-6-phosphate]/[glucose-6-phosphate]	0.25 \pm 0.03		0.87 \pm 0.12	0.30 \pm 0.01	0.33 \pm 0.06	
[Glucose-6-phosphate]/[glucose-1-phosphate]	—		—	27.3 \pm 3.6	—	
[2-Phosphoglycerate]/[3-phosphoglycerate]	0.20 \pm 0.06		0.19 \pm 0.02	0.14 \pm 0.01	0.18 \pm 0.01	
[α -Glycerophosphate]/[dihydroxyacetone phosphate]	3.85 \pm 0.40		0.28 \pm 0.22	0.61 \pm 1.08	8.52 \pm 1.95	
[ATP]/[AMP]/[ADP]	0.97 \pm 0.17		0.31 \pm 0.17	2.80 \pm 0.70	0.90 \pm 0.17	

from the mean concentration of phosphorylated intermediates, adenine nucleotides, IMP and P was increased at 10 min and at 40 min on perfusion with D-fructose. Livers freeze-clamped *in vivo* contained 13.8 μmol of total phosphorus/g whereas those perfused with fructose contained 16.3 $\mu\text{mol/g}$ at 10 min and 40 min. The total phosphorus content returned to normal (14.3 $\mu\text{mol/g}$) at 80 min. The transient increase in total phosphorus content was due to an increase in the concentration of phosphorylated interme-

diates. The values for total phosphorus content are approximate because a few intermediates such as UTP and UDP-glucose that decrease in concentration during treatment with D-fructose (9) were not determined in the freeze-clamped livers. However the contribution of these intermediates is small. At 10 min and 40 min the increase in total phosphorus content represents an uptake of 19.58 μmol of phosphorus from the perfusion medium by 8 g of liver. This hepatic phosphorus uptake may account for the transient

Table II. Balance of fructose metabolism in the perfused rat liver

The amounts of products formed represent the sum of metabolites in medium and liver. The quantities of most other intermediates in medium and liver (listed in Table I) were negligibly small and have therefore been omitted from the calculation of the balance.

Expt. no.	Liver wt. (g)	Duration of perfusion (min)	Products formed					Sum of products (expressed in C ₃ units) (μmol)
			Fructose removed (μmol)	Fructose-1-phosphate (μmol)	Lactate (μmol)	Pyruvate (μmol)	Glucose (μmol)	
1	9.38	40	1048	63	894	30	701	1075
2	7.51	40	756	79	456	45	559	828
3	8.00	40	1063	89	707	40	749	1182
4	10.90	40	1049	68	696	87	703	1180
5	10.14	60	1323	37	1419	83	898	1660
6	9.30	80	1175	54	748	78	930	1367
7	9.95	80	1074	14	845	67	907	1227

lowering of serum P_i in human subjects during intravenous D-fructose infusion (25)

The concentrations of lactate and pyruvate increased steadily and in parallel. Lactate reached an average concentration of 4.3 μmol/g after 80 min which is much higher than the concentrations obtained in the absence of fructose when, under the same conditions, mM was rarely exceeded. The [lactate]/[pyruvate] ratio remained fairly constant throughout the perfusion period at a value near 10

The metabolite ratios were all of the order expected for equilibrium with the exception of the mass action ratio of the adenylate kinase system (ATP)/(ADP)² which was outside the expected range after 10 and 40 min. Adenylate kinase is taken to be located in the outer mitochondrial membrane and to act slowly upon the cytoplasmic adenine nucleotides (4, 11). As ketohexokinase, AMP deaminase and 5-nucleotidase are cytoplasmic enzymes, the depletion of the adenine nucleotides after fructose loading is expected to concern the cytoplasm in the first instance. If the adenine nucleotides that remain after fructose loading were mainly mitochondrial, some deviations of the mass action ratio from equilibrium would be understandable.

Balance between fructose disappearance and metabolites formed

The sum of the products formed from fructose as calculated in Table II, was somewhat greater (up to 10 % at 40 min and up to 23 % at 80 min) than the amounts of fructose removed. This surplus is explained by the inclusion of some glucose formed from glycogen. The calculations indicate that the greater part of the fructose removed was converted into glucose. It is unlikely that

Table III. Rates of metabolite changes in the medium of the perfused rat liver

The initial rates of metabolite changes during perfusion with D-fructose (initial concentration 10 mM) were calculated from a plot of each metabolite in the total perfusion medium versus time. The results are means \pm S.E.M. for seven observations. The experiments were the same as those in Table II.

Metabolite changes	Rate (μmol/min per g wet wt.)
Fructose removed	2.63 \pm 0.25
Glucose formed	1.87 \pm 0.22
Lactate formed	1.74 \pm 0.16
Pyruvate formed	0.15 \pm 0.01

there were significant quantities of metabolites other than those listed in Table I, and the complete oxidation of fructose could at most have contributed 10 / to the removal of fructose because the rate of oxygen consumption was $2.6 \mu\text{mol/min}$ per g of liver. This could have oxidised $0.43 \mu\text{mol}$ of fructose/min whereas the rate of removal was $2.63 \mu\text{mol/min}$ per g of liver.

The values for the main initial rates of fructose removal and glucose, lactate and pyruvate production in the perfusion medium (Table III) were obtained for the balance experiments of Table II from a plot of metabolites in the total medium versus time. Linearity usually extended for about 60 min. The values for the rates confirm that virtually all the fructose removed during the earlier period of perfusion is accounted for by the formation of glucose, lactate and pyruvate.

A comparison of the fructose content in the freeze-clamped perfused liver with that

in the perfusion medium at the time of clamping shows that a gradient existed for this metabolite between the medium and the liver (Table IV). The concentrations in the liver were several fold lower. The values for fructose content of the freeze-clamped liver have been corrected for the fructose content and volume of the entrapped medium by using the value 22.2 / obtained by Krebs et al. (23) for the extracellular space, which consists mainly of the liver vascular system and its contents.

The initial rate of fructose removal was $2.63 \mu\text{mol/min}$ per g wet wt. of liver and the existence of the concentration gradient implies that the rate of penetration of fructose is less than the rate of its metabolism.

Cause of accumulation of fructose 1 phosphate

The results outlined above can serve as a basis for the calculation of the rates of fruc

Table IV Fructose content of liver and perfusion medium

The values for fructose content of the liver have been corrected for the fructose content of the extracellular space using the figure 22.2 / (23). The bulk of the extracellular space was the perfusion medium within the hepatic vascular system.

Time (min)	Expt. no.	Perfusion medium ($\mu\text{mol/ml}$)	Fructose content		
			Perfused liver		Fructose in medium/ Fructose in tissue (corrected) ratio
			Uncorrected ($\mu\text{mol/g}$ wet wt.)	Corrected for fructose content and volume of the extracellular space ($\mu\text{mol/g}$ wet wt.)	
10	1	8.53	8.23	4.29	2.0
	2	3.06	4.04	3.89	2.8
	3	7.33	3.03	1.63	4.0
	4	8.74	3.13	1.53	5.7
40	1	2.60	0.56	< 0.01	
	2	3.05	0.82	0.18	16.9
	3	4.52	1.38	0.50	9.0
	4	2.73	0.53	< 0.01	
80	1	1.90	0.38	< 0.01	
	2	0.23	0.01	< 0.01	
	3	1.17	0.29	< 0.01	

Table II. Balance of fructose metabolism in the perfused rat liver

The amounts of products formed represent the sum of metabolites in medium and liver. The quantities of most other intermediates in medium and liver (listed in Table I) were negligibly small and have therefore been omitted from the calculation of the balance.

Expt. no.	Liver wt. (g)	Duration of perfusion (min)	Fructose removed (μ mol)	Fructose-1-phosphate (μ mol)	Products formed			Sum of products (expressed in C ₆ units) (μ mol)
					Lactate (μ mol)	Pyruvate (μ mol)	Glucose (μ mol)	
1	9.38	40	1048	62	394	30	701	1075
2	7.51	40	786	79	456	45	559	889
3	8.00	40	1065	89	707	40	749	1182
4	10.80	40	1049	66	696	67	702	1160
5	10.14	80	1338	37	1419	53	898	1660
6	9.30	80	1175	54	748	78	930	1397
7	9.93	80	1074	14	845	67	907	1327

lowering of serum P in human subjects during intravenous D-fructose infusion (28)

The concentrations of lactate and pyruvate increased steadily and in parallel. Lactate reached an average concentration of 4.3 μ mol/g after 80 min which is much higher than the concentrations obtained in the absence of fructose when, under the same conditions, 2 mM was rarely exceeded. The [lactate]/[pyruvate] ratio remained fairly constant throughout the perfusion period at a value near 10

The metabolite ratios were all of the order expected for equilibrium with the exception of the mass action ratio of the adenylate kinase system, (ATP)/(AMP)/(ADP)² which was outside the expected range after 10 and 40 min. Adenylate kinase is taken to be located in the outer mitochondrial membrane and to act slowly upon the cytoplasmic adenine nucleotides (4, 11). As ketohexokinase, AMP deaminase and 5-nucleotidase are cytoplasmic enzymes, the depletion of the adenine nucleotides after fructose loading is expected to concern the cytoplasm in the first instance. If the adenine nucleotides that remain after fructose loading were mainly mitochondrial, some deviations of the mass action ratio from equilibrium would be understandable

Balance between fructose disappearance and metabolites formed

The sum of the products formed from fructose, as calculated in Table II, was somewhat greater (up to 10 % at 40 min and up to 23 % at 80 min) than the amounts of fructose removed. This surplus is explained by the inclusion of some glucose formed from glycogen. The calculations indicate that the greater part of the fructose removed was converted into glucose. It is unlikely that

Table III. Rates of metabolite changes in the medium of the perfused rat liver

The initial rates of metabolite changes during perfusion with D-fructose (initial concentration 10mM) were calculated from a plot of each metabolite in the total perfusion medium versus time. The results are means \pm S.E.M. for seven observations. The experiments were the same as those in Table II.

Metabolite changes	Rate (μ mol/min per g wet wt)
Fructose removed	2.63 \pm 0.23
Glucose formed	1.87 \pm 0.23
Lactate formed	1.74 \pm 0.16
Pyruvate formed	0.15 \pm 0.01

Table V Inhibitions by cell constituents of the rat liver aldolase reactions in tris hydrochloric acid buffer at pH 7.4

The preparation of the liver extract and details of the assay are given in ref. 46. The effect of Mg^{2+} was tested when it was found that Mg^{2+} can abolish the inhibition of activity on fructose-1-phosphate by ATP. The substances marked with an asterisk contained, according to the makers, between 1 and 6 % of the mono- and di-phosphates.

Percentage inhibition of aldolase reactions with

Substances tested (1 mM)	Fructose-1-phosphate (0.067 mM)	Fructose-1 phosphate (0.067 mM) MgCl (7.5 mM)	Fructose-1,6-diphosphate (0.033 mM)
ATP	63	0	0
ADP	42	17	0
AMP	84	44	0
ITP	68	38	0
IDP	86	71	0
DMP	90	74	0
GTP	82	37	0
GDP	76	53	0
GMP	80	57	0
UTP	88	13	0
UDP	89	38	0
UMP	67	60	0
CTP	78	0	0
CDP	81	14	0
CMP	57	37	0
L-α-Glycerophosphate	35	37	20
2-Phosphoglycerate	44	37	6
3-Phosphoglycerate	41	38	0
Phosphoenolpyruvate	31	36	36
Glucose-1-phosphate	78	30	0
Glucose-6-phosphate	32	27	15
Fructose-6-phosphate	22	30	18
P	29	25	0
D-Glyceraldehyde	49	48	0

gave values ranging from 0.14 to 0.66 (average 0.35 mM fructose-1 phosphate). This is lower than the K_m values found for rabbit liver aldolase 0.8 mM (37) and 2 mM (43).

The effect of some intermediates and inhibitors on the adenine nucleotide depletion and fructose 1 phosphate accumulation after fructose loading

The effect of the addition of adenine, aspartate, P and dipyrimidole (a pyrimido-pyrimidine compound) on the adenine nucleotide depletion induced by fructose was investigated in perfused livers (Table VII). The hepatic contents of adenine nucleotides,

Table VI Effect of substrate and inhibitor concentration of the inhibition by IMP of rat liver aldolase fixation of fructose 1 phosphate

The preparation of the liver extract and method of the assay are given by Woods et al. (46). Corrections have been made for blank without fructose 1-phosphate.

Concentration of fructose- 1-phosphate	Rate of fixation of fructose- 1-phosphate ($10^3 \times \Delta E$ in 8 min \pm 25 °C)		
	Control (no IMP)	0.5 mM IMP	1.0 mM IMP
0.25	118	30	17
0.5	145	53	27
1.0	163	82	52
2.5	180	118	89
5.0	196	142	121
10.0	190	153	138

fructose-1-phosphate formed by the tissue compared with that accumulated. Within 10 min 8.7 μmol of fructose-1-phosphate had accumulated per g of tissue. Since the capacity of hexokinase is very low in relation to that of ketohexokinase (16) most of the other products formed from fructose (at a collective rate of 2.63 $\mu\text{mol}/\text{min}$ per g, Table III) must have passed through the stage of fructose-1-phosphate about 30 % of the fructose-1-phosphate formed during the first 10 min had accumulated, and although the rate of fructose removal remained constant for another 35–50 min the concentration of fructose-1-phosphate did not continue to increase. This fact, together with the observation that the potential capacity of the aldolase is high enough to split more than 2.6 mol of fructose-1-phosphate/min per g (Table XIII) suggests that the accumulation of fructose-1-phosphate is caused by an inhibition of the aldolase that is overcome when the concentration of fructose-1-phosphate rises. The following experiments bear out this hypothesis.

A systematic search for inhibitors of the aldolase reaction among liver constituents (Table V) shows that several phosphorylated metabolites inhibit liver aldolase and that the fission of fructose-1-phosphate is much more sensitive to inhibitions than the fission of fructose-1,6-disphosphate. However the concentration of the majority of potential inhibitors is not sufficiently high in the liver to be effective. Possible exceptions are IMP, AMP, GMP, UMP, CMP and α -glycerophosphate. The tri- and di-phosphates of adenosine, guanosine, uridine and cytidine are powerful inhibitors in the absence of Mg^{2+} but since these substances are present in the tissue mainly as Mg^{2+} chelates they cannot be major inhibitors *in vivo*. The concentrations of GMP, UMP and CMP are probably too low for them to be major inhibitors so that IMP and α -glycerophosphate must be taken to be the main agents that affect the activity of aldolase because the sum of the

concentrations of GTP, GDP, GMP, UTP, UDP, UMP, CTP, CDP, CMP is less than 1.5 $\mu\text{mol}/\text{g}$ of liver (6, 20, 38). Adenosine, inosine, adenine, hypoxanthine, fructose and glucose were not inhibitory.

The extent of the inhibition of fructose-1-phosphate fission by a particular inhibitor depends on the presence of other inhibitory phosphorylated compounds in the incubation medium. In general the inhibitions recorded in Table V were not additive. Measurements of the rate of fission in solutions designed to imitate the essential metabolite concentrations of the liver (as recorded in Table I) show that the inhibition in a liver environment equivalent to perfusion with 10 mM fructose for 10 min is 62 % and that equivalent to 40 min is 22 % provided that the fructose-1-phosphate concentration is at the normal value of 0.2 $\mu\text{mol}/\text{g}$. Increasing the fructose-1-phosphate concentration to 3 $\mu\text{mol}/\text{g}$ decreased these inhibitions to 44 % and 16 % respectively at the two times. The main inhibitor under these conditions is IMP. Although P_i alone is inhibitory this inhibition is not additive to that by IMP. In further experiments not recorded in the Tables combinations of the four purine and pyrimidine mono-, di- and tri-phosphates at physiological concentrations had no significant effects on the inhibition of fructose-1-phosphate fission by IMP and α -glycerophosphate.

Detailed information on the degree of inhibition of fructose-1-phosphate fission by IMP at different substrate and inhibitor concentrations is given in Table VI. A plot of $1/v$ against $1/s$ (26) from the data in Table VI indicates a competitive nature of the inhibition and a plot of $1/v$ against I according to Dixon (13) from this and other experiments gave an average K_i of 0.1 mM—IMP. The K_m for the substrate from results in Table VI (graphical methods of Schwimmer (39) and Dixon (13)) was approximately 0.18 mM—fructose-1-phosphate but other experiments

Dipyramidole inhibits cardiac muscle adenosine deaminase (EC 3.5.4.4) *in vivo* (7). The effect of this compound on adenine nucleotide depletion was investigated by perfusing livers with a medium which contained 10 mM D-fructose and 0.1 mM dipyramidole. It was noted that these livers were the distinct yellow colour of dipyramidole when freeze-clamped which suggested that the drug was concentrated by the liver. The total adenine nucleotide content fell to 60 % of that *in vivo* at 10 and 40 min, thus adenosine deaminase may play a part in the adenine nucleotide depletion. In a separate experiment it was found that dipyramidole at a concentration of 0.1 mM did not inhibit rat liver aldolase reactions.

Hepatic metabolite contents during anaerobic perfusion in the presence of fructose

Rat liver slices are known to convert added fructose readily into lactate when the conditions are anaerobic if the rat has been well fed (12, 34, 35, 36). The rates of glycolysis in isolated perfused livers from well fed rats are much greater than those measured in tissue slices, and are increased by the addition of D-fructose, the anaerobic changes with regard to carbohydrate metabolism being the sum of the metabolism of endogenous glycogen and the added fructose (47). The changes in glycolytic metabolites in rat liver after ischaemia *in situ* have been investigated by Hems and Brosnan (17). Under the conditions of their experiments rates of glycolysis during the first two minutes after severance of the circulation were about half of those observed in the perfused organ (3).

In the experiments reported below the contents of adenine nucleotides, IMP and the intermediates of glycolysis were determined in freeze-clamped livers perfused under anaerobic conditions when the rates of gly-

colysis are rapid and comparable to those observed in tissues which have a high glycolytic capacity (brain, embryonic tissue, testicle leucocytes, tumours). The intermediates were determined during anaerobic perfusion when the substrate for glycolysis was hepatic glycogen and during perfusion with a medium containing 10 mM D-fructose. The livers were freeze-clamped after 40 min of perfusion had elapsed when the time-course of glycolysis was linear.

Intermediates of glycolysis in livers perfused in the absence of added substrate The contents of liver metabolites after 40 min of anaerobic perfusion are shown in Table VIII. Comparison with the values found in livers from fed rats freeze-clamped *in vivo* after cervical dislocation shows the following

Adenine nucleotides inorganic phosphate and IMP The content of ATP decreased to 19 % of that found *in vivo*. The ADP also decreased to 67 % of the *in vivo* level. The fall in ATP and ADP content was accompanied by a two-fold rise in the AMP content. The total adenine nucleotide content fell to 40 % of control mainly due to the fall in ATP. The P content did not change significantly. The mean total phosphorus content of the liver calculated from the values obtained for the content of the phosphorylated intermediates, adenine nucleotides, IMP and P determined in each liver was 16.8 $\mu\text{mol/g}$ wet wt., which is higher than that calculated for livers of fed rats freeze-clamped *in vivo* (12.8 $\mu\text{mol/g}$ wet wt.). The calculated total liver phosphorus content was only approximate because all phosphorylated intermediates were not determined, and under anaerobic conditions, nucleotides other than the adenine nucleotides may have fallen in content. Lactate and pyruvate. There was a five-fold rise in the lactate content of the anaerobic liver. The pyruvate content fell by half and the [lactate]/[pyruvate] ratio rose from

Table VIII. Contents of intermediary metabolites in the anaerobic perfused rat liver

Livers from well fed rats were freeze-clamped after 40 min of perfusion under anaerobic conditions in the presence of 1 mM HCN. The data refers to livers perfused without added substrate and to livers perfused in the presence of D-fructose (initial concentration 10 mM). The results are compared with those obtained for livers of well fed animals freeze-clamped *in vivo* following cervical dislocation. The metabolite contents are mean \pm S.E.M.

	Metabolite content ($\mu\text{mol/g}$ wet wt.)		
	Liver not perfused	Livers perfused with no added substrate	Livers perfused with D-fructose
Time of perfusion (min)	0	40	40
No. of observations	4	5	5
ATP	2.22 ± 0.07	0.43 ± 0.03	0.54 ± 0.02
ADP	0.79 ± 0.06	0.53 ± 0.03	0.71 ± 0.04
AMP	0.26 ± 0.03	0.55 ± 0.04	0.49 ± 0.02
Total adenine nucleotides	3.27 ± 0.12	1.51 ± 0.10	1.74 ± 0.03
DAMP	0.12 ± 0.03	0.22 (2)	0.52 ± 0.04 (4)
Pi	4.25 ± 0.25	3.92 ± 0.46	4.44 ± 0.47
D-Glyceraldehyde	—	—	0.71 (3)
α -Glycerophosphate	0.13 ± 0.10	0.26 ± 0.56	4.30 ± 0.43
Fructose-1-phosphate	0.23 (2)	—	5.54 ± 0.78
Free fructose	< 0.01	—	2.58 ± 0.20
Free glucose	7.22 ± 0.77	9.48 (3)	7.15 ± 1.73
Lactate	0.79 ± 0.06	4.81 ± 0.74	5.82 ± 0.43
Pyruvate	0.06 ± 0.01	0.06 ± 0.01	0.11 ± 0.01
Fructose-1,6-diphosphate	0.02 ± 0.002	0.02 ± 0.003	0.03 ± 0.005
Glucose-6-phosphat	0.33 ± 0.03	0.32 ± 0.05	0.31 ± 0.06
Fructose-6-phosphate	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
Glucose-1-phosphate	< 0.01	< 0.01	< 0.01
Phosphoenolpyruvate	0.16 ± 0.02	0.04 ± 0.003	0.03 ± 0.01
2-Phosphoglycerate	0.04 ± 0.01	0.03 ± 0.005	0.05 ± 0.01
3-Phosphoglycerate	0.26 ± 0.06	0.10 ± 0.01	0.16 ± 0.02
Glyceraldehyde-3-phosphate	0.006 ± 0.0002	0.01 ± 0.0007	0.012 ± 0.0025
Dihydroxyacetone phosphate	0.04 ± 0.003	0.12 ± 0.006	0.16 ± 0.021
Citrate	0.45 ± 0.06	0.09 (2)	0.19 ± 0.02
α -Oxoglutarate	0.11 ± 0.003	0.03 (2)	—

a value of 96 in fed livers freeze-clamped *in vivo* to 101 in livers perfused under anaerobic conditions.

Hexose phosphates Among the hexose phosphates the contents of FDP G-6-P and F-6-P all rose but the changes were small.

3-Carbox compounds The triose phosphates both rose, dihydroxyacetone phosphate three-fold, while glyceraldehyde-3-phosphate all most doubled. The contents of phosphoenolpyruvate 2-PGA and 3-PGA all fell. The most striking change was the accumulation of α -glycerophosphate which rose seventy fold to $9.26 \mu\text{mol/g}$ wet wt.

These changes can be summarised by stating that above the triose phosphate level the intermediates of the glycolytic sequence all rose in content and those below fell.

Other intermediates. The glucose content rose by about one-third to $9.48 \mu\text{mol/g}$ wet wt. and the contents of the TCA cycle intermediates citrate and α -oxoglutarate both fell.

Metabolite ratios The metabolite ratios for some of the reactions of glycolysis calculated from the measured contents of intermediates in livers freeze-clamped *in vivo* and after 40 min of anaerobic perfusion (Table IX) indicate that the reactions catalysed by phos-

Table IX. Metabolite ratios in perfused rat liver

The metabolite ratios have been calculated from the contents in Table VIII for livers from well fed rats freeze-clamped *in vivo* and after 40 min of perfusion under anaerobic conditions with no added substrate and with D-fructose (initial concentration 10 mM). The ratios have been calculated in the direction of lactate formation except for the triose phosphate isomerase reaction. The adenylate kinase equilibrium has been calculated in the direction of ATP formation. Apparent equilibrium constants quoted were taken from the following references (10, 37, 38, 44).

Measured ratio	Livers perfused under anaerobic conditions			Enzyme
	Fed liver <i>in vivo</i>	No added substrate	D-fructose (10 mM)	
(A)	(B)	(C)	(D)	K'
Lactate / Pyruvate	9.97 ± 0.90	100.94 ± 16.33	52.91 ± 8.50	Lactate dehydrogenase (EC 1.1.1.27)
(α-Glycerophosphate)/ Dihydroxyacetone phosphate	3.35 ± 0.40	76.44 ± 8.75	28.20 ± 2.93	α-Glycerophosphate dehydrogenase (EC 1.1.1.8)
(Glucose-6-phosphate) [ADP]/ [Glucose] [ATP]	0.01 (37)	0.03 (37)	0.04 ± 0.007	Hexokinase (EC 2.7.1.1)
(Fructose-6-phosphate) / [Glucose-6-phosphate]	0.25 ± 0.03	0.56 ± 0.01	0.28 ± 0.03	Phosphofructokinase (EC 2.7.1.3)
(Fructose-1,6-bisphosphate) [ADP]/ [Fructose-6-phosphate] [ATP]	0.10 ± 0.01	0.48 ± 0.04	0.45 ± 0.10	Phosphofructokinase (EC 2.7.1.11)
(Glyceraldehyde-3-phosphate)/ [Fructose-1,6-bisphosphate]	0.09 ± 0.03 × 10 ⁻⁴ M	0.43 ± 0.08 × 10 ⁻⁴ M	0.18 ± 0.07 × 10 ⁻⁴ M	Triose phosphate isomerase (EC 5.3.1.1)
(Dihydroxyacetone phosphate)/ [Glyceraldehyde 3-phosphate]	8.90 ± 0.44	12.70 ± 1.40	14.23 ± 1.52	Triose phosphate isomerase (EC 5.3.1.1)
(3-Phosphoglycerate)/ [Phosphoenolpyruvate]	0.50 ± 0.06	0.30 ± 0.07	0.32 ± 0.04	Phosphoenolpyruvate carboxykinase (EC 4.1.3.1)
(Phosphoenolpyruvate)/ [Pyruvate] [ATP]/ [ATP] [ADP]	3.84 ± 0.21	1.61 ± 0.24	0.97 ± 0.07	Pyruvate kinase (EC 2.7.1.40)
(Phosphoenolpyruvate) [ADP]/ [ATP] [ADP]	1.76 ± 0.46	1.09 ± 0.22	2.60 ± 1.06	Adenylate kinase (EC 2.7.4.3)
[ADP] ²	0.97 ± 0.17	1.03 ± 0.24	0.55 ± 0.06	0.94—1.03 (3) depends on [Mg ²⁺]

phoglucose isomerase, phosphoglycerate mutase, enolase and adenylate kinase were close to equilibrium while those catalysed by hexokinase, phosphofructokinase and pyruvate kinase were far from equilibrium, both *in vivo* and under anaerobic conditions. The triose phosphate isomerase and fructose diphosphate aldolase reactions were not in equilibrium *in vivo* but under anaerobic conditions moved towards equilibrium. The initial rate of lactate production in these experiments was $2.56 \pm 0.24 \mu\text{mol/min/g wet wt.}$, which is very similar to that reported from this laboratory (47).

Intermediates of glycolysis in livers perfused with D-fructose

Livers from well fed rats were perfused in the presence of 1 mM HCN with a medium containing D-fructose (initial concentration, 10 mM). The livers were freeze-clamped after 40 min of perfusion and the perfusion medium was sampled at intervals to determine the time course of changes in the concentration of lactate, pyruvate, glucose and D-fructose.

The contents of intermediary metabolites in fructose-perfused livers are shown in Table VIII and comparison with values obtained for livers freeze-clamped *in vivo* shows the following

Adenine nucleotides and inorganic phosphate and IMP The total adenine nucleotide content decreased to 53 % of the *in vivo* value, this was mainly due to the fall in ATP content to 25 % of the *in vivo* value, the ADP content decreased a little to 90 % of the *in vivo* value and the AMP content rose about two-fold. The P_i content did not change significantly. The fall in the total adenine nucleotide content was accompanied by a three-fold rise in the content of IMP to $0.52 \mu\text{mol/g wet wt.}$ This is higher than

the IMP content during anaerobic perfusion in the absence of added substrate and suggests that deamination of AMP to give IMP was more rapid in the presence of D-fructose.

Lactate and pyruvate The content of lactate rose seven fold to $5.82 \mu\text{mol/g wet wt.}$, which is higher than the content found in livers from well fed rats perfused under identical conditions in the absence of D-fructose. The pyruvate content rose 13 % to $0.11 \mu\text{mol/g wet wt.}$ and the [lactate]/[pyruvate] ratio rose to 52.9 which is half the value found in anaerobic livers perfused in the absence of D-fructose.

Other phosphorylated intermediates. The content of the phosphorylated intermediates were much the same as those found in livers perfused in the absence of D-fructose in that the intermediates above the triose phosphate level of the glycolytic sequence rose while those below fell. The one exception to this general statement was the content of 3-phosphoglycerate, which rose 12 % to $0.65 \mu\text{mol/g wet wt.}$ in livers perfused with D-fructose. The triose phosphates both rose dihydroxyacetone phosphate four fold and glyceraldehyde-3-phosphate two-fold.

Fructose-1 phosphate rose twentyfour fold to $5.54 \mu\text{mol/g wet wt.}$ and α -glycerophosphate to $4.40 \mu\text{mol/g wet wt.}$

Other intermediates The glucose content did not change significantly and the free fructose content at the time of freeze-clamping was $2.68 \mu\text{mol/g wet wt.}$ The D-glyceraldehyde content was $0.71 \mu\text{mol/g wet wt.}$ and the citrate content fell to $0.19 \mu\text{mol/g wet wt.}$ The balance and rates of anaerobic D-fructose metabolism. The sum of the products found during the anaerobic perfusion of livers from well fed rats is shown in Table X. The balance refers to the perfusion medium and liver in experiments where the livers were

Table X. Balance of D-fructose metabolism in the anaerobic perfused rat liver

The amounts of products found represent the sum of metabolites in medium and liver found after 40 min of perfusion in the presence of 1mM HCN and an initial concentration of 10 mM D-fructose. The quantities of most other intermediates in medium and liver (listed in Table VIII) were small and have been omitted from the calculation of the balance. Results are all expressed as μmol .

Liver wt.	Fructose removed	Products found					Sum of products (expressed in C ₅ units)
		F 1 P	α -GP	Lactate	Pyruvate	Glucose	
7.35	845	42	38	1351	30	534	1304
8.42	575	65	46	1043	15	425	1043
8.12	528	31	31	869	7	763	1248
9.45	751	38	41	1525	7	1370	2095
8.85	582	54	25	1026	18	528	1121

freeze-clamped after 40 min. If the assumption is made that all the fructose passes through the F 1 P stage, some 7.3 / of the fructose metabolised accumulated as F 1 P in the liver. The initial rates of metabolic change obtained for the experiments (Table XI) are of the same order of magnitude as those given by Woods and Krebs (47) for livers from well-fed rats perfused under identical conditions.

Metabolite ratios The metabolite ratios for some of the reactions of glycolysis (Table IX) were similar to those measured in livers freeze-clamped in the absence of fructose. The reactions catalyzed by hexokinase, phosphofructokinase and pyruvate kinase were far from equilibrium; the triose phosphate isomerase and fructose diphosphate aldolase reaction ratios moved closer to equilibrium under anaerobic conditions in the presence of D-fructose.

The distribution of metabolites between liver and perfusion medium

Comparison of the metabolite concentrations in the medium with those in the liver after correction for the metabolite content and volume of the entrapped medium, as de-

scribed above, shows that gradients exist for glucose, fructose and lactate (Table XII). In both groups of experiments the hepatic glucose content was higher than that in the medium, the difference being 3.48 mM in the absence of added substrate and 2.46 mM in the presence of fructose. The concentration of fructose in the medium was 3.04 mM greater than that in the liver. The lactate content of the liver, the site of production, was lower than that in the medium. In the absence of added substrate this gradient was small (0.88 mM) and was not statistically significant.

Table XI. The rates of metabolic changes during anaerobic perfusion

The initial rates of metabolic changes during anaerobic perfusion of livers from well fed rats perfused with D-fructose (initial concentration 10 mM) were calculated from plot of total metabolites in the perfusion medium versus time. The results are means \pm S.E.M. for five observations. The experiments were the same as those in Table.

Metabolite change	Initial rate ($\mu\text{mol}/\text{min}/\text{g}$ wet wt.)
Fructose removed	2.24 ± 0.24
Glucose formed	2.07 ± 0.37
Lactate formed	3.29 ± 0.23
Pyruvate formed	0.05 ± 0.006

Table XII. Metabolite concentrations in freeze-clamped liver and perfusion medium

The values for the metabolite content of liver have been corrected for the metabolite content and volume of the extracellular space as described in the text. The data refers to livers from well-fed rats freeze-clamped after 40 min of anaerobic perfusion in the presence of 1mM HCN. The results are mean \pm S.E.M. with the number of observations in parentheses.

Condition	Metabolite	Metabolite concentration (mM)		Metabolite ratio in medium
		Perfusion medium	Freeze-clamped liver (corrected)	Metabolite in tissue (corrected)
Livers perfused in the absence of added substrate	Glucose	6.59 (3)	10.07 (2)	0.65
	Lactate	5.38 ± 0.53 (3)	4.40 ± 0.57 (3)	1.20
	Pyruvate	< 0.01 (3)	0.05 ± 0.008 (3)	—
Livers perfused with D-fructose (initial concentration 10 mM)	Fructose	8.00 ± 0.40 (3)	1.96 ± 0.20 (3)	2.55
	Glucose	8.20 ± 1.05 (3)	7.72 ± 2.00 (3)	0.66
	Lactate	7.61 ± 0.70 (3)	5.02 ± 0.39 (3)	1.32
	Pyruvate	0.10 ± 0.03 (3)	0.11 ± 0.01 (3)	0.91

($p > 0.2$) but it rose to 2.59 mM in the presence of fructose, a difference in concentration which was statistically significant ($p < 0.01$)

DISCUSSION

Adenine nucleotide depletion after fructose loading

The experiments confirm that when the rat liver is loaded with fructose, either by intravenous injection of fructose or the addition of fructose to the medium perfusing the isolated organ, there is a rapid loss of hepatic adenine nucleotides (27-32) accompanied by a large accumulation of fructose-1 phosphate. At 10 min after fructose loading some 36 % of the original adenine nucleotides were recovered as IMP.

The hepatic changes after fructose loading can be explained on the basis of the properties of the enzymes of fructose metabolism and of adenine nucleotide degradation. The primary step as suggested by Maenpää et al. (27) is the rapid phosphorylation of

fructose to fructose-1 phosphate, catalysed by ketohexokinase. This decreases the concentration of ATP and P_i , both of which are essential in stabilizing AMP and therefore the total adenine nucleotide content of the liver. They inhibit the enzymes that cause the virtually irreversible degradation of AMP. P_i inhibits AMP deaminase (30) and at high concentrations 5-nucleotidase (40) which is also inhibited by ATP (2). When the inhibition of AMP deaminase becomes less effective the hepatic IMP concentration rises and AMP and IMP are dephosphorylated with the eventual formation of uric acid and allantoin.

In addition to fructose, loading the liver with glycerol (8-48) or xylitol (48) also results in adenine nucleotide depletion and the accumulation of large amounts of α -glycerophosphate. The activation of hepatic AMP degrading enzymes as outlined above might be expected to occur during the hepatic metabolism of any substrate involving a rapid phosphorylation step and the accumulation of a phosphorylated intermediate. This would result in a lowering of the hepatic P content

Table XIII. Properties of enzymes of fructose metabolism in rat liver

The results refer to normal well-fed rats but the strains and diets used by different authors were not identical. The assays of ketohexokinase in this laboratory were carried out as described by Adelman et al. (1) and those of ketose-1 phosphate aldolase as described previously (46)

	Ketohexokinase	Ketose-1 phosphate aldolase	References
Maximal rates ($\mu\text{mol/min per g}$ of tissue) \pm 25°C and pH 7.4—7.6, mean \pm S.D. and number of observations	$\left\{ \begin{array}{l} 3.12 \\ 3.14 \pm 0.81 \text{ (7)} \\ 2.20 \pm 0.39 \text{ (8)} \end{array} \right.$	$\left\{ \begin{array}{l} 3.40 \pm 0.36 \text{ (3)} \\ 1.63 \pm 0.11 \text{ (5)} \end{array} \right.$	$\left\{ \begin{array}{l} (1) \\ (46) \\ (16) \end{array} \right.$
K_m (mM) (mean, S.D. and no of observations)	$\left\{ \begin{array}{l} 0.2 - 0.5 \\ 0.4 \end{array} \right.$	$\left\{ \begin{array}{l} 0.35 \pm 0.18 \text{ (8)} \end{array} \right.$	$\left\{ \begin{array}{l} (1) \\ (31) \\ (46) \end{array} \right.$

because the sequestration of phosphate as the intermediate is greater than the amount of P liberated by the dephosphorylation of the adenine nucleotides. This hypothesis is confirmed by the finding that loading with dihydroxyacetone, which does not lead to adenine nucleotide depletion, does not result in hepatic accumulation of dihydroxyacetone phosphate or other phosphorylated intermediate (9, 48).

The accumulation of fructose-1 phosphate

The accumulation of fructose-1 phosphate after fructose loading has been known since 1942 (22) the reasons for the accumulation have hitherto not been understood. It could not be explained on the basis of the relative activities of ketohexokinase and ketose-1 phosphate aldolase because under the conditions in the liver *in vivo* the activities of the two enzymes are about the same (Table XIII). After fructose loading, when the concentration of free fructose is lower than that of fructose-1 phosphate, the activity of ketohexokinase may be expected to be lower than those of ketose-1 phosphate aldolase. The finding that the IMP which accumulates strongly inhibits ketose-1 phosphate aldolase fully accounts for the accumulation of fructose-1-phosphate.

The anaerobic metabolism of fructose

The rapid metabolism of fructose and the hepatic accumulation of fructose-1-phosphate during the anaerobic perfusion (Table VIII) indicate that the conditions are favourable for hepatic fructokinase activity. Dihydroxyacetone phosphate and D-glyceraldehyde, the products of the fructose-1-phosphate aldolase reaction, did not accumulate to any great extent and thus the reactions subsequent to this step must have been rapid. The phosphorylation of D-glyceraldehyde to glyceraldehyde-3-phosphate by the triokinase (EC 2.7.1.28) reaction is favoured because of the low K_m for D-glyceraldehyde in this reaction (0.01 mM, ref. 41), and some 90 % of the D-glyceraldehyde formed from fructose in the liver is metabolised in this way (41). An alternative pathway of D-glyceraldehyde metabolism is conversion to 2-phosphoglycerate by oxidation to glyceraldehyde dehydrogenase (EC 1.2.1.3) and subsequent metabolism via glyceralate kinase (EC 2.7.1.3). In the absence of added substrate the hepatic 2-phosphoglycerate content fell in contrast it rose in the fructose-perfused anaerobic livers. This may have been due to the metabolism of some D-glyceraldehyde via the glyceralate pathway.

Pyruvate kinase is activated by fructose-1-phosphate at concentrations which occur in rat liver during perfusion with 10 mM fructose (14). Anaerobic lactate formation from fructose (via pyruvate kinase) is more rapid than that from endogenous glycogen (47). The maximum rate of lactate formation from fructose in the perfused liver is up to 4 $\mu\text{mol/min/g}$ wet wt. (47) and the activity of pyruvate kinase when assayed at physiological concentrations of phosphoenolpyruvate is of the same order (14). It is therefore to be postulated that the activation of pyruvate kinase by fructose-1 phosphate is essential for maximal rates of anaerobic fructolysis.

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EFFECT OF FRUCTOSE ON CELLULAR RESPIRATION IN PERFUSED RAT LIVER

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Abstract. 1 The regulation of energy metabolism is reviewed with special reference to the metabolism of fructose. Fructose metabolism in liver by-passes the main regulatory step of glycolysis, the formation of fructose-6-phosphate. Therefore the overall regulation of energy metabolism is possibly adjusted in a new way. The by-pass and the high activity of liver fructokinase cause metabolic alterations characterized by an accumulation of fructose-1-phosphate and depletion of inorganic phosphate and adenine nucleotides in the liver.

2. The effects of fructose on the redox state of nicotinamide nucleotides and mitochondrial flavoproteins and cytochromes were studied by surface fluorometry and transmission spectrophotometry in isolated perfused rat liver.

Oxygen consumption was inhibited and nicotinamide nucleotides were reduced concomitant with phosphate depletion. The spectrophotometry of intact tissue showed inhibition of electron transport; the first phosphorylation site of the mitochondrial respiratory chain.

The fructose-induced alterations in the cellular redox state can be interpreted as acceptor control for inorganic phosphate during the metabolism of fructose. The role of the metabolism of glyceraldehyde in these redox changes is discussed.

The metabolism of fructose in the liver has some unusual characteristics: 1) it takes place at a high rate compared with glucose metabolism (22), 2) it involves several metabolic crossroads not shared by glucose metabolism (4, 13, 15), 3) this suggests that the control mechanisms of fructose metabolism may be different from those of glucose metabolism. Fructose is mainly phosphorylated to fructose-1-phosphate, which is directly attacked by aldolase B (fructose-1-phosphate aldolase) to yield D-glyceraldehyde and dihydroxyacetone phosphate (4, 20). Thus, the rate of fructose catabolism in the liver is not influenced by phosphofructokinase which is considered to be the most important regulatory enzyme in glycolysis (27). It might be expected that this lack of anaerobic control of fructose breakdown would be reflected in a typical pattern of effects on aerobic energy metabolism. We shall refer to the latter as cellular respiration. This includes the terminal oxidations in the tricarboxylic

acid cycle and respiratory chain with the energy conservation system.

The aerobic fate of the trioses formed from fructose in the liver is probably similar to the fate of the three-carbon compounds formed in glycolysis. The formation of glycerophosphate (2) from fructose is not reflected in the respiratory rate because the activity of the mitochondrial glycerophosphate dehydrogenase is very low (14).

Since fructose evades the major regulatory step of glycolysis, the mutual regulation of glycolysis and respiration would be expected to become seriously unbalanced. This normal integration of glycolysis and respiration is shown by the Pasteur and Crabtree effects (3, 18). The main mechanisms in the control of respiration are the acceptor control of the mitochondrial respiratory chain for ADP and inorganic phosphate (3), the adenylate control of the tricarboxylic acid cycle (1) and the control of the tricarboxylic acid cycle by the redox state of mitochondrial nicotinamide adenine nucleotides (25). Obviously the overall control of energy metabolism involves a complex network of these mechanisms.

This communication reviews briefly the fructose-induced changes in cellular respiration. It also describes some effects of fructose on the intracellular redox state and on regulation by the mitochondrial electron transport carriers. These were studied by sensitive fluorometric and spectrophotometric methods in intact tissues.

EFFECT OF FRUCTOSE ON THE OXYGEN CONSUMPTION OF THE LIVER

When 5 to 10 mM fructose is added to the perfusate of a liver perfusion, there is a rapid initial stimulation of oxygen consumption lasting 2 to 3 minutes. This stimulation is followed by an inhibition reaching a maximum after 6 to 7 minutes. In liver from a fed rat respiration drops to 70% of the steady state value (9) but in starved rat liver

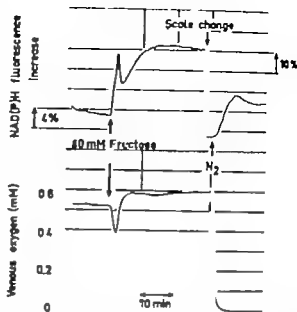


Fig. 1 Effect of fructose on the redox state of liver nicotinamide nucleotides. NAD(P)H fluorescence (upper curve) was recorded by surface measurement of an isolated perfused rat liver. Perfusion medium: Krebs-Ringer bicarbonate buffer pH 7.4, 35 flow rate 20 ml/min. Fructose (10 mM) was added to the perfusion medium at the injection port before the oxygenator to avoid oxygen tension artifacts. Oxygen concentration in the outlet cannula in the inferior caval vein was monitored with Clark type oxygen electrode. Arterial oxygen concentration was kept constant at 1.01 mM, except under the cycle of anoxia induced by changing the gas phase (95% O_2 , 5% CO_2) to 95% N_2 , 5% CO_2 at the time indicated by N_2 . The rat fasted 20 hrs before the experiment. The fluorometer used fibre optics for both the excitation and emission light (7). Excitation light wavelength (365 nm) was isolated from a low-pressure mercury arc lamp and modulated at 50 Hz. The multiplier phototube was guarded against excitation light by a Kodak Wratten 2A filter. Zero suppression was with the excitation light intensity as a reference signal. Percentage numbers refer to the initial fluorescence of the tissue.

the inhibition is smaller (Fig. 1). Occasionally inhibition persists, but usually it fades after 10 minutes. The pattern of the respiratory inhibition is also affected by the thyroid state of the animal (11).

EFFECT OF FRUCTOSE ON THE INTRA-CELLULAR REDOX STATE

Both the stimulatory and inhibitory phases are accompanied by reduction of nicotinamide nucleotides. The time course of this effect is easily demonstrated by measuring the NAD(P)H fluorescence of whole tissue (Fig. 1). There is a small reoxidation at the time of the transition from stimulation to inhibition of respiration. This redox change is reflected in the NAD-linked redox substrate pairs, for instance there is a rise in the lactate/pyruvate (L/P) concentration ratio (2, 12, 19). The time course of the changes in L/P ratio has not been studied in detail by rapid sampling. However samples taken by Raivio et al. (18) at 1, 2, 5 and 10 min. showed an L/P ratio maximum at 5 min. This is the opposite to the oxidative effect which the sorbitol dehydrogenase and glyceraldehyde reductase (alcohol dehydrogenase) enzymes could be expected to exert. If these two enzymes are functioning in the metabolism of fructose, their effects must be swamped by other effects, such as NADH formation in glycolysis or perhaps effects at the level of mitochondrial oxidations.

We tested the latter possibility by studying the redox changes of mitochondrial respiratory carriers under these same conditions (9). The redox state of the fluorescent mitochondrial flavoproteins shifted towards oxidation during the stimulation of respiration and towards reduction during the inhibition of respiration (Fig. 2). The fluorescent flavoprotein of intact liver tissue has been identified as the lipolic dehydrogenase (8, 24), i.e. the flavoprotein component of the α -oxoacid dehydrogenase complex. This flavoprotein is in equilibrium with the mitochondrial NADH/NAD pool, and thus is an indicator of the redox state of mitochondrial nicotinamide adenine nucleotides in intact tissue (24). Comparison of the traces of Figs. 1 and 2 shows a dissociation between the redox transitions

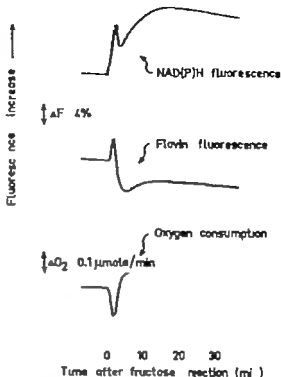


Fig. 2 Effect of fructose on the respiration and redox state of the nicotinamide nucleotides and flavoproteins in isolated perfused rat liver. Experimental conditions as in Fig. 1. Fructose (10 mM) was added to the perfusion medium at time 0. Flavoprotein fluorescence was measured by using the 438 nm mercury line for fluorescence excitation. The multiplier phototube was guarded against excitation light by a broad-band filter peaking at 532 nm with half intensity band width of 57 nm. Oxygen concentration in the venous cannula was monitored with an oxygen electrode. The two upper curves are means of three experiments, the lowest of six experiments. Shaded area represents mean \pm SEM.

of cytosol (as reflected in NADH fluorescence) and mitochondria (as reflected in flavin fluorescence). This is somewhat puzzling because at least during the ethanol induced redox change of NADH/NAD the changes in cytosol and mitochondria are roughly parallel (11, 24). However this dis-

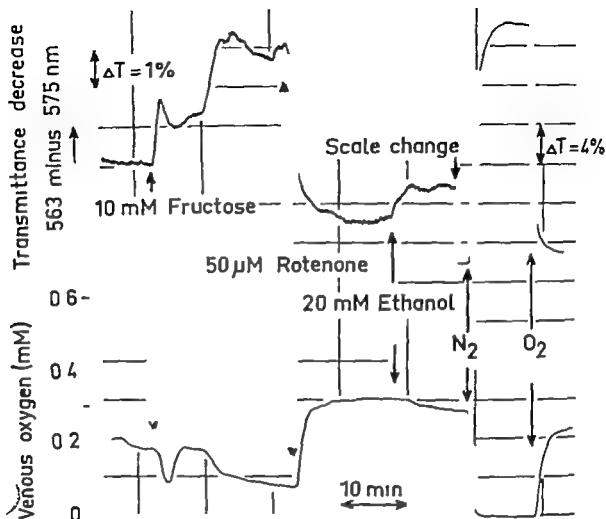


Fig. 3 Effect of fructose on the redox state of cytochrome *b* and respiration in an isolated perfused rat liver. Experimental conditions as in Fig. 1. Fructose (10 mM) was added at the time indicated. Thereafter the cytochrome was completely oxidized by adding rotenone (50 μ M) and completely reduced by anoxia (as in Fig. 1). The upper curve represents the difference of transmittances at 563 and 575 nm recorded by dual wavelength spectrophotometer. An upward deflection means reduction of cytochrome *b*. Oxygen concentration (lower curve) in the venous cannula was monitored with an oxygen electrode.

sociation is only evident during the rapid initial changes. The long term effects in both compartments are towards reduction.

EFFECTS OF FRUCTOSE ON THE MITOCHONDRIAL RESPIRATORY CHAIN

The behaviour of the mitochondrial redox state suggests a control on the oxygen side of the mitochondrial NADH/NAD pool. This is highly probable since the spectrophotometrically measurable redox changes in the mitochondrial cytochrome chain showed a control point at this level (9, 10). The most clearcut redox changes were in cytochrome *b*. These changes were almost a mirror image of the changes in the redox state of the fluorescent flavoproteins (Fig. 3). The redox transitions of cytochrome *cc*₁ and cytochrome *aa* were much more difficult to interpret (9).

GENERAL DISCUSSION OF THE CONTROL OF FRUCTOSE METABOLISM

The results described so far can be explained by inhibition of respiration at the level of the mitochondrial respiratory chain during fructose metabolism.

The respiratory inhibition is most likely to be caused by the changes in the intracellular adenylate compounds and inorganic phosphate brought about by fructose (2, 17-19). This finding was originally made by Maenpää et al. (17) and will be discussed elsewhere in this Symposium. The experiments have been repeated with perfused rat livers. The results were similar in principle the only difference being the smaller change in adenine nucleotides in spite of a drastic change in inorganic phosphate (8, 18). The changes in inorganic phosphate kept in pace with the transitions of the respiratory rate (11). It is likely that the depletion of phosphate is caused by a rapid glycolytic and oxidative phosphorylation of the ADP formed in the phosphorylation of fructose to fructose-1-phosphate (3). One tempting explanation is that the respiratory inhibition is an expression of a mutual control of glycolysis and respiration. In other words, this is a phenomenon very similar to the Crabtree effect observed in ascites tumour cells (5). In fact, phosphate control of glycolysis and respiration has been demonstrated in ascites tumour cells (16, 20).

The physiological significance of the effects described remains to be established. The results may only be valid for parenteral administration of fructose. The absorption of fructose from the intestine is quite slow judging by the blood fructose levels found after large oral doses (30). Intravenous fructose decreases serum phosphate levels in man (26). This might indicate that the phosphate control is not specific to one species.

The redox state of nicotinamide adenine nucleotides after fructose administration be-

haves in accordance with the results of Silero et al. (25). They found that the enzyme profile of the liver and the kinetic parameters of the enzymes involved in the metabolism of glyceraldehyde indicate that the phosphorylation of glyceraldehyde by thio kinase is the main pathway. The isotope distribution data obtained with isotopically labelled fructose also argue against the significance of the reductive pathways (reduction by alcohol dehydrogenase to glycerol) (21).

The phosphate control of respiration after fructose loading seems to be one of the few situations in which mitochondrial respiratory control can be demonstrated in intact tissue. For this reason it may develop into a tool for investigating the degree of coupling between oxygen consumption and energy conservation in intact mammalian tissue.

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FRUCTOSE AND PURINE METABOLISM

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Abstract. The interactions between fructose and purine metabolism are reviewed. Hyperuricemia and increased production of uric acid after fructose administration have been documented in man as well as in the rat. These effects appear to be dependent on the rapid phosphorylation of fructose, mainly in the liver. This results in depletion of intracellular ATP and inorganic phosphate. As consequence, the adenylate deaminase reaction is deinhibited and increased breakdown of adenine nucleotides to uric acid ensues.

Some considerations of the hazards involved in the clinical use of fructose are presented, and other possible points of interaction between fructose and purine metabolism are discussed.

Some kind of interaction between fructose and purine metabolism was suggested by the observation that in patients with hereditary fructose intolerance and in normal individuals, a rapid intravenous infusion of fructose resulted in hyperuricemia (16). In subsequent studies, the biochemical basis as well as the clinical implications of this phenomenon have been elucidated, and an alteration of purine nucleotide degradation by fructose has been demonstrated. It is not yet clear whether other aspects of purine

metabolism, e.g. *de novo* synthesis, are also affected.

The hyperuricemic response to fructose occurs rapidly in normal individuals, with a peak at 15 min after an intravenous injection of 0.5 g/kg (16). The simultaneous increase in the urinary excretion of urate rules out the possibility of renal retention as the basis for the phenomenon (16), even though blood lactate levels also increase rapidly. In accord are the studies of Fox and Kelley (7), which indicated an unaltered clearance of uric acid during fructose-induced hyperuricemia. The renal tubular secretion of urate is known to be inhibited when blood lactate levels are elevated by infusing sodium lactate (25), but this mechanism is not responsible for the rapid rise in serum urate after fructose administration.

Fructose-induced hyperuricemia thus seems to be due to increased formation of uric acid, either through *de novo* synthesis or through breakdown of preformed purine nucleotides. Evidence for the latter possibility has been obtained by studying rats, in which fructose-induced hyperuricemia and hyperallantoinemia also occur (16).

An intravenous injection of 1 mmole of fructose causes a rapid depletion of the adenine nucleotides of rat liver with a maximal decrease of 50 %/ 5 min after the injection (18, 20). This is mainly due to a decrease of ATP levels, and it is accompanied by a more rapid and marked drop in hepatic inorganic phosphate (P_i) concentrations. Both changes are largely normalized by 30 min after the injection. On the other hand, a marked accumulation of fructose-1-phosphate occurs and persists for a more prolonged period (3, 9, 15). All these alterations can also be demonstrated in an isolated perfused rat liver preparation, when fructose is added to the perfusion medium (24). They as well as fructose-induced hyperuricemia, can be explained on the basis of the known regulatory behavior of certain enzymes of fructose and nucleotide metabolism.

Fructose is rapidly phosphorylated by ketohexokinase (1, 10, 24) utilizing ATP and resulting also in a decrease in P_i levels. These changes allow an increased rate of the AMP deaminase reaction, which is normally inhibited by P_i (17) and by ATP (21). It is probable that the alternate route of AMP degradation through 5-nucleotidase also increases, because this enzyme is normally inhibited by ATP (2). A marked increase in IMP concentration occurs (24) and is probably responsible for an accentuated sequestration of P_i as fructose-1-phosphate because IMP has been shown to inhibit fructose-1-phosphate aldolase (24). IMP itself is further broken down to inosine, hypoxanthine, xanthine and finally uric acid. If xanthine oxidase activity is inhibited by allopurinol, fructose-induced degradation of adenine nucleotides is manifested in an increased excretion of the oxipurines hypoxanthine and xanthine (7).

The postulated sequence of events explains the specificity of the effects of fructose on purine metabolism. Only fructose and L-sorbose are phosphorylated by ketohexokinase at a rapid rate (1) and therefore other hexoses are without effect (20). Ketohexo-

kinase is present only in the liver, kidney and intestinal mucosa (1), explaining the lack of effect of fructose on the ATP levels in rat heart (20).

The importance of increased breakdown of preformed purine nucleotides in the causation of fructose-induced hyperuricemia is supported by rough calculations on the basis of liver perfusion experiments. The quantitative increase in allantoin production agreed closely with the estimated loss of adenine nucleotides from the intact liver (20). However, a primary stimulation of *de novo* purine synthesis has not been ruled out as a contributing mechanism. In any case, a secondary increase in *de novo* synthesis is to be expected, because the depletion of adenine nucleotides would tend to release the feedback inhibition exerted by these compounds on the rate-limiting enzyme of purine synthesis, 5-phosphoribosyl 1-pyrophosphate amidotransferase. Preliminary data indicate that the incorporation of ^{14}C -glycine into urinary uric acid is increased as a result of fructose infusion, but it has not been possible to evaluate whether this is a primary or a secondary effect (19).

The clinical implications of fructose-induced hyperuricemia deserve comment, because fructose is commonly used in intravenous fluid therapy and is a common constituent of the diet. As discussed above, the elevation of serum and urinary uric acid seems to be a consequence of the breakdown of adenine nucleotides mainly in the liver. In patients undergoing abdominal surgery a 50% decrease in liver ATP and ADP levels has in fact been demonstrated by serial biopsy in the course of a 30 min infusion of fructose but not of glucose (4). Interference with protein synthesis is a known manifestation of such a depletion (8, 16), whereas other possible consequences are largely unexplored. In any case infusion of fructose at rates producing hyperuricemia can be considered potentially harmful. Even at lower rates, elevation of blood lactate levels and resulting

changes in acid-base balance must be taken into account (3, 14).

The injection of a single dose of 0.5 g/kg of fructose was followed by a marked increase in serum and urinary urate in children (18) and an oral dose of 1 g/kg had an unequivocal effect in normal and gouty adults (23). On the other hand, a continuous fructose infusion at the rate of 0.5–0.7 g/kg/hr apparently does not influence serum urate levels in adults (6, 11, 22) but at the rate of 1–1.5 g/kg/hr a hyperuricemic response has been demonstrated (11, 14). It is apparent that a significant amount of calories can be supplied intravenously in the form of fructose without provoking significant hyperuricemia. In post-operative patients receiving fructose at the rate of 0.3 g/kg/hr a slight increase in urinary uric acid excretion was noted in comparison to patients receiving glucose (12) but this difference was not impressive in comparison to the catabolic effect of surgical trauma *per se*. On the basis of animal experiments it appears that the increased breakdown of phosphorylated adenine derivatives by anoxia and by fructose administration may be additive in action (13).

It is also apparent that a marked elevation of blood lactate levels occurs even when fructose is administered in amounts not producing changes in serum uric acid levels (22). Therefore, from the clinical standpoint acid-base considerations may outweigh possible interference with purine metabolism in evaluations of the usefulness of fructose.

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FRUCTOSE AND LIVER PROTEIN SYNTHESIS

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Abstract Intravenous administration of 5 mmoles per kg of D-fructose to rats causes a transient but strong inhibition of incorporation of ^{14}C leucine into liver protein. The inhibition is maximal 2 to 3 minutes after the fructose injection and is practically over 25 minutes later. The inhibition of ^{14}C -leucine incorporation is closely correlated with the depletion of adenosine triphosphate (7). Liver polysome profiles are essentially unaltered 5 minutes after the administration of fructose, but at 15 and 30 minutes gradual and extensive disaggregation of large polysomes is observed. Changes in the hepatic levels of free magnesium are thought to be involved in the alterations of the polysome profiles after fructose administration.

As has been shown by us (7, 8) and by others (1, 4, 13), intravenous or intraperitoneal administration of large doses of fructose rapidly depletes liver adenosine triphosphate in laboratory animals and man. This process probably interferes with most of the liver functions. This report will discuss its effects on liver protein synthesis. It will show that both amino acid incorporation and the size distribution of the hepatic ribosome popula-

tion are changed after a single intravenous injection of fructose.

MATERIAL AND METHODS

The test animals were female albino rats weighing approximately 0.2 kg. They were fasted over night prior to the experiments. Incorporation of amino acid into liver protein was examined by giving an intravenous 2-minute pulse of 2 μCi of ^{14}C DL-leucine (specific activity 55.2 mCi/mmmole) at various times relative to an intravenous injection of 5 mmoles per kg of D-fructose. Liver samples were rapidly frozen at the times stated by the rapid-freezing technique and the radioactivity in hot trichloroacetic acid treated protein was determined as described previously (7).

Liver polysome profiles were studied 0, 5, 15, and 30 minutes after injection of fructose. Liver samples were rapidly removed, weighed and homogenized in Tris buffer (25 mM Tris, 25 mM NaCl, and 5 mM MgCl_2) containing 0.14 M sucrose and 100 $\mu\text{g}/\text{ml}$ of heparin, with seven strokes in Potter Elvehjem type homogenizer Deoxycholate and Triton X 100 were added to final concentration of 1 per cent each, and the tissue was homogenized with two more strokes (8). The resulting 10 per cent liver homogenate was centrifuged for 5 minutes at $30,000 \times g$. The supernatant (0.2 ml) was layered over 0.5 to 1.5 M

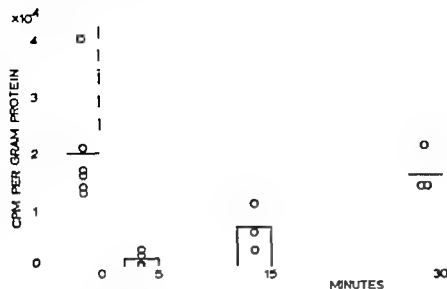


Fig. 1 Effect of intravenous injection of 5 mmoles per kg of D-fructose on incorporation of ^{14}C -leucine into liver protein *in vivo*. Rats were given an intravenous 3-minute pulse of ^{14}C -leucine at designated times before and after fructose administration. From Mäenpää, Räsäio and Kekkonen (7). Copyright 1968 by the American Association for the Advancement of Science.

linear sucrose gradient made in the above buffer plus 40 $\mu\text{g}/\text{ml}$ of heparin, and centrifuged for 80 minutes at $192,000 \times g_{\text{max}}$ in the SW 50.1 rotor. The gradients were analyzed by passing them through a 3-mm Gilford flow cell connected to a Zeiss PMQ II recording spectrophotometer. Transmittance at 60 nm was recorded and converted to absorbance units. The absorbance units were multiplied by 5 to correct for the 3-mm light path.

RESULTS AND DISCUSSION

As shown in Fig. 1 incorporation of ^{14}C -leucine into liver protein is strongly inhibited 2 to 5 minutes after fructose administration being less than 10 per cent of the control value. The fructose effect is brief and by 30 minutes the value is again the same as in the controls. Comparison of the time

courses of the changes in ATP levels and in ^{14}C -leucine incorporation indicates a close correlation between these two variables (7). The exact mechanism by which fructose influences amino acid incorporation is unknown, but the parallel alterations in ATP and in incorporation of the label suggest that it may be due to blocked aminosacylation. In some other test systems the levels of cellular ATP and incorporation of radioactive amino acid into protein show an analogous correlation, although the time course may differ (3, 11, 12). It should be pointed out that the fructose effect is very rapid.

Fig. 2 shows the sedimentation characteristics of hepatic total ribosome population before and at various times after the administration of fructose. The dose was identical to that used previously. The size distribution of the ribosomes remains essentially unchanged at the time of maximal inhibition of ^{14}C -leucine incorporation. However at 15 and at 30 minutes, a significant gradual disaggregation of the large polysomes into monosomes is observed which suggests that the ribosomes are incapable of initiating a new cycle of peptide synthesis. Although the precise relationship between fructose administration and altered hepatic polysome profiles remains to be determined,

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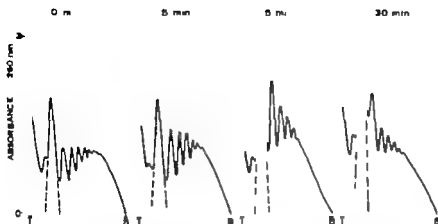


Fig. 2. Liver polysome profiles before and at various times after injection of fructose as in Fig. 1. Polysomes were prepared and analyzed as described in the text. Direction of sedimentation in the sucrose gradients is from left to right and location of the monosome peak in each gradient is indicated by interrupted lines.

changes in free magnesium levels may be involved. A large part of the magnesium present in the cell is normally bound to ATP. After fructose injection when liver ATP is depleted, part of the magnesium may be released into the blood. In this connection it is interesting to note that in a patient with hereditary intolerance to fructose serum magnesium levels were increased after fructose administration (6). When the rebound of liver ATP takes place, the newly formed ATP may lower the free magnesium level still further by binding part of it. Thus, the situation after fructose administration may bear a resemblance to that demonstrated *in vitro* when the so-called runoff ribosomes are released from polyribosomes by incubation under conditions of amino acid incorporation with less than 7 mM Mg^{2+} (2, 3).

Here, the total ribosome population was studied. Administration of fructose may also influence the distribution of ribosomes between free and membrane-bound forms, since magnesium is thought to play an important role in the ribosome-membrane interaction (10). A recent ultrastructural study supports this view (14).

These data indicate that parenteral administration of large doses of fructose to rats has multiple effects on liver protein synthesis. Since fructose has been shown to lower the hepatic ATP level in man also (1) caution is called for when intravenous fructose is used for various reasons in man.

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METABOLISM OF FRUCTOSE AND GLYCERALDEHYDE IN THE ISOLATED PERFUSED PIG LIVER

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Abstract. Isolated pig livers were perfused under near physiological conditions, i.e. use of pigs blood, perfusion via both arterial and portal system, and constant pH (7.4). Fructose uptake and the production of a number of metabolites were measured in relation to the fructose concentration in the perfusion medium. A Michaelis type kinetics was found with V_m about 3 μ moles/g liver \times min and K_m about 8 mM. Fructose-1 phosphate was observed to accumulate as consequence of the inhibition of the aldolase step, presumably by glyceraldehyde, ADP and IMP. Sorbitol formation is of small importance under our experimental condition.

During continuous fructose infusion the concentration of glyceraldehyde was low (less than 0.5 mM). Therefore the metabolism of glyceraldehyde was studied separately. Measurement of the reaction products glycerol and glycerate as function of the glyceraldehyde concentration support the assumption that glyceraldehyde is metabolized by several pathways.

Measurement of acid production during metabolism of either fructose or D-glyceraldehyde suggests

that considerable accumulation of acids occur in the liver as only a minor part of the titration value is accounted for as the organic acids measured, of which lactic acid is the quantitatively most important.

Only 20—30 % of the carbon of fructose and glyceraldehyde is accounted for through formation of lactate and glucose. The rest is oxidized or accumulates in some form in the liver.

The oxygen consumption during fructose metabolism was increased about 30 %, and the lactate/pyruvate ratio showed small, but reproducible changes.

The metabolism of fructose in the liver still presents some problems, which need clarification, viz.

1. How does the metabolism of fructose depend on the concentration of fructose in the blood?
2. What is the relative contribution of the various pathways suggested to account for the fate of glyceraldehyde? (cf. Fig. 1).
3. How does fructose (and glyceraldehyde)

The team responsible for this investigation further includes K. Tennesen, F. Vælle Hansen and K. Winkler.

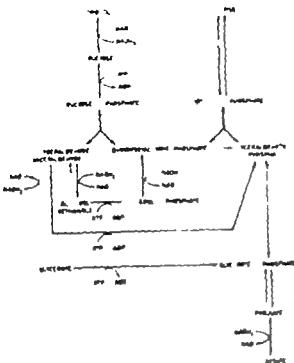


Fig. 1 Metabolic pathways of fructose in the liver

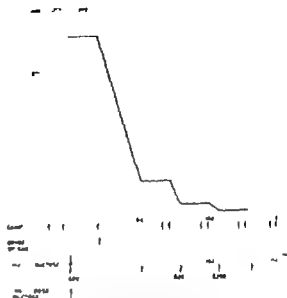


Fig. 2 The calculated time course of an experiment with declining fructose elimination rate. The infusion of fructose was calculated as $\mu\text{moles/g} \cdot \text{min}$. Periods with constant infusion were 31 minutes. After each steady state period the infusion was stopped for the period necessary to reach the next steady state fructose concentration.

Influence the metabolic condition of the liver (redox state oxygen consumption phosphorylation potential, etc.)?

Some of these problems were approached by means of perfusion of the isolated pig liver. This preparation was chosen primarily because of the importance of this system in the treatment of hepatic coma by extra-corporal perfusion, but also because the large volume of circulating blood permits the simultaneous determination of a very large number of metabolites.

METHODS

Pig livers were perfused with heparinized pig blood diluted with physiological saline to a hematocrit value of about 18. Perfusion was performed simultaneously through the portal vein and the hepatic artery at physiological pressures. The pH of the circulating medium was kept at 7.4 by titration with NaHCO_3 as considerable amounts of acid are produced in these experi-

ments. The anoxic period involved in establishing the artificial circulation was in general 3–4 min at 32°C . The rate of perfusion was kept constant and the pressure in both arterial and portal system continuously recorded. The blood was equilibrated with 95% O_2 – 5% CO_2 . Details of the perfusion system have been published (11).

The material comprises 11 experiments. Some of the physiological parameters are listed in Table 1. The experiments were started after an equilibration period of about 60 min, including a period in which pressure and temperature were unchanged for at least 30 min, and no acid production was recorded by the titrator. In order to obtain steady state conditions the substrates (fructose or glyceraldehyde) were infused at constant rates for about 30 min. The infusion rate necessary to establish a desired concentration in the system was calculated from preceding experiments, in which a single large dose was given and the elimination followed until the substrate was completely removed. Fig. 2 shows an experimental plan in which the theoretical fructose concentrations were calculated from such an experiment with a single dose of fructose. Fig. 3 shows the levels actually obtained.

Table I. Physiological parameters of the present experiments (mean \pm SD ($n = 11$))

Liver weight (kg)	1.191 \pm 0.077	Arterial pressure (mm Hg)	123 \pm 23
Blood volume (l)	3.86 \pm 0.59	Portal pressure (mm Hg)	12.9 \pm 4.4
Total flow (l/min)	1.22 \pm 0.31	Transsinusoidal resistance (mm Hg/l \times min)	11.0 \pm 4.2
Arterial flow (l/min)	0.17 \pm 0.14	Bile flow (ml/min)	360 \pm 1.0

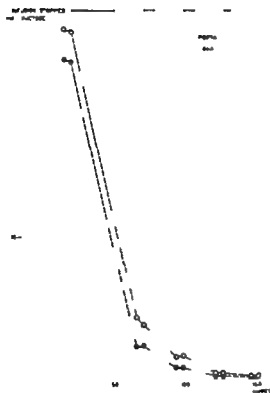


Fig. 3. The actual fructose concentrations obtained in the experiment referred to in Fig. 2.

RESULTS

Initial steps in fructose elimination

Fig. 4 shows the rate of fructose elimination as a function of the fructose concentration in the portal blood (affluent blood). The elimination rate was calculated both from the simultaneous concentration difference over the liver and from the change in con-

centration with time of the pool of circulating blood.

Three processes might be considered as candidates for the rate limiting step in fructose uptake by the liver: 1) transport of fructose across the cellular membrane, 2) reduction to sorbitol and 3) phosphorylation to fructose-1-phosphate (or 6-phosphate).

The possibility that some transport process is rate limiting does not appear likely as our limited biopsy material suggests that equilibrium is established between tissue and circulating medium.

The formation of sorbitol, which seems to be a dead end in fructose metabolism, is of small importance under our experimental conditions (Table II). The concentration of sorbitol in the medium is about 3–4 % of the fructose concentration under steady state conditions indicating equilibrium during the steady periods (2), but not in the period when the fructose concentration falls precipitously.

Phosphorylation to fructose-1-phosphate has repeatedly been described in other species (8, 6, 13). Also in the pig a considerable accumulation occurs as seen in Table III, indicating that ketohexokinase is of importance also in the pig. The concentration of fructose-6-phosphate was not significantly increased in the biopsies. When our elimination curve was converted to a Lineweaver-Burk plot a fair approximation to Michaelis-Menten kinetics was observed. The kinetic parameters for the elimination are given in Table IV together with results on crude preparations from pig liver. A 10 times higher K_m value was found for the perfused liver. However, the measurements of K_m reported in the literature

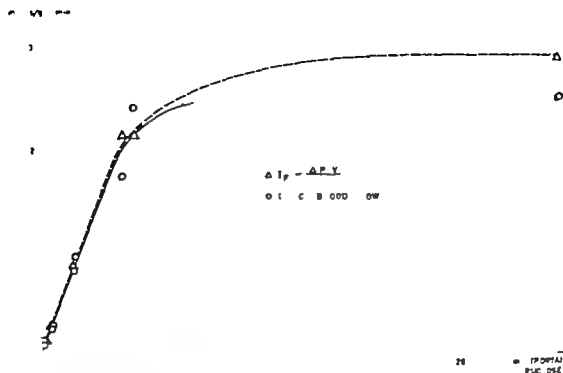


Fig. 4. The elimination rate of fructose versus the fructose concentration in the affluent medium calculated from the experiment referred to in Fig. 1. Two methods of calculation have been employed: Δ = Infusion rate corrected for simultaneous accumulation of substrate in the medium, O = concentration difference between affluent and effluent medium times total flow.

rate (0.5 mM; see ref. 18) as well as our own measurements were made in the virtual absence of ADP. The presence in the liver of this substance, which is known to modify the ketohexokinase reaction, may be the explanation for the high apparent K_m found *in vivo*. Interference from hexokinase at high fructose concentrations appears less likely but cannot be excluded.

Table II. Sorbitol concentration in the affluent blood during increasing fructose elimination. At 90 minutes the fructose infusion was stopped.

Time (min)	Portal concentrations (mM)		Sorbitol/ Fructose
	Fructose	Sorbitol	
-5	0.00	0.073	0.00
40	4.63	0.065	0.01
75	10.4	0.070	0.03
90	14.1	0.50	0.04
110	3.70	0.320	0.12

The aldolase step

The splitting of fructose-1-phosphate into glyceraldehyde and dihydroxyacetone phosphate is apparently a bottleneck in the metabolism of fructose in liver tissue, since fructose-1-phosphate may accumulate in very large quantities. This step was followed through measurement of glyceraldehyde. Fig. 3 shows a characteristic feature of the experiments. Immediately after fructose infusion the glyceraldehyde concentration increases up to about 1 mM and then drops off

Table III. Concentrations of fructose 1 phosphate in the liver biopsies. The figure below the bottom line is a mean value from 3 pigs not given fructose

Fructose-1 P (μ moles/g liver)	Fructose (mM, Cava)
1.1	0.10
1.3	0.19
5.1	2.5
6.3	2.0
3.6	2.6
0.7 (n = 3)	0.0

rapidly while the fructose concentration is still high. After nearly complete removal of the fructose, the liver may liberate glyceraldehyde again as seen in Fig. 6. The obvious interpretation of these results is that the aldolase step is strongly regulated, as was in fact shown by Woods et al. (13) who observed inhibition by D-glyceraldehyde, AMP and IMP. Initially after administration of a large dose of fructose the concentration of the three substances mentioned will be low and accordingly the aldolase will function at full capacity resulting in an outburst of D-glyceraldehyde. This in itself together with the increasing concentrations of AMP and IMP inhibits the cleavage of fructose-1-phosphate, which accordingly accumulates.

When the supply of free fructose is exhausted the fructose-1-phosphate accumulated will again give rise to glyceraldehyde, as AMP and IMP are rapidly further metabolized (presumably to allantoin). This may



Fig. 5. Concentration of D-glyceraldehyde during stepwise reduction of fructose concentration from 25 mM. The lower part of the figure is derived from the experiment shown in Figs. 2, 3, 4.

explain the secondary bursts of glyceraldehyde described above.

A consequence of these findings is that under steady state conditions the concentration (found to be less than 0.5 mM) and the output of glyceraldehyde will be low and apparently dependent on the previous history of the liver. We therefore performed some

Table IV. The elimination kinetics of fructose in the isolated perfused pig liver compared to the kinetic constants of the pig liver ketohexokinase (Mean values \pm S.E.M.) The results for ketohexokinase were obtained on high speed supernatant directly (V_m) or after acid treatment (K_m) to remove sorbitol dehydrogenase

	K_m (mM)	Maximum elimination rate (μ moles/g \times min)
Perfused pig liver	5.9 ± 0.4 (n = 4)	2.9 ± 0.4 (n = 4)
Pig liver ketohex kinase	0.5 (n = 2)	1.9 ± 0.4 (n = 4)

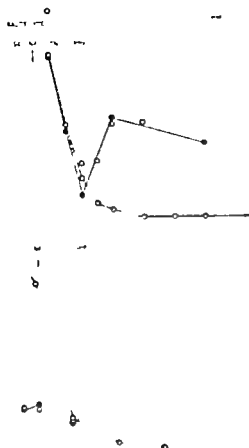


Fig. 6. Release of D-glyceraldehyde coincident with cessation of fructose elimination.

experiments with infusion of D-glyceraldehyde in the perfused pig liver in order to examine the fate of glyceraldehyde at higher concentrations.

D-glyceraldehyde metabolism

Several metabolic pathways may be open to glyceraldehyde (see Fig. 1): oxidation by



Fig. 7. Elimination rate of D-glyceraldehyde as function of the concentration in the afferent medium.

means of NAD-requiring aldehyde dehydrogenase to glycerate, reduction to glycerol by NAD or NADP requiring enzymes, phosphorylation to glyceraldehyde-3-phosphate by triokinase and condensation with dihydroxy acetone phosphate to fructose-1-phosphate. Fig. 7 shows the rate of glyceraldehyde removal as a function of the concentration in the circulating medium. It is striking that the removal of glyceraldehyde is very slow ($0.2-1 \mu\text{moles/g min}$) at concentrations corresponding to those in the steady state fructose experiments. The various plateaus may correspond to individual processes, but it should be mentioned that the curve cannot be constructed simply by addition of a series of Michaelis curves. However Fig. 8, in which the concentrations of lactate, glycerate and glycerol at different glyceraldehyde concentrations are given, suggests that new processes

Table 1. Kinetic constants of some pig liver enzymes involved in D-glyceraldehyde metabolism (Mean \pm S.E.M.) D-Gald D-glyceraldehyde D-Ga D-glycerate

Enzyme	Coenzyme	Substrate	K_m (mM)	V_{max} (U/g)
Alcohol dehydrogenase	NADH	D-Gald	18 ± 2	14 ± 3 (n = 4)
Alcohol dehydrogenase	NADPH	D-Gald	5.6 (n = 7)	4.3
Aldehyde dehydrogenase	NAD	D-Gald	0.16 (n = 7)	0.64 ± 0.07 (n = 4)
Glycerate kinase	ATP	D-G	—	0.17 ± 0.03 (n = 3)

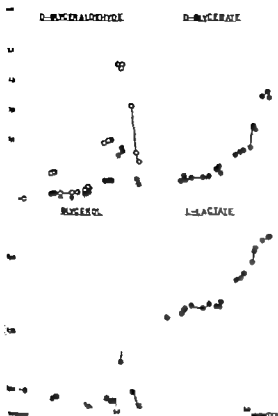


Fig. 8. Concentrations of D-glycerate, glycerol and L-lactate in the effluent medium in an experiment in which D-glyceraldehyde was infused to maintain different concentration levels in the effluent medium.



Fig. 9. Concentrations of D-glyceraldehyde glycerol and D-glycerate in the effluent and effluent media in an experiment in which fructose was infused to maintain different concentration levels in the effluent medium.

ses prevail as the concentration of aldehyde is increased, although the figures can only be taken as rough indicators of the rate of the primary processes, as these substances may be further metabolized. The lack of glycerol formation except at very high concentrations of D-glyceraldehyde is in agreement with the high apparent K_m for ADH (both NADH and NADPH requiring) found with crude preparations from pig liver (Table V). It is noteworthy that the activity of glycerate kinase is very low (Table V) in the pig in contrast to some other species (10). In agreement with this finding an accumulation of glycerate is always observed whether glyceraldehyde or

fructose is used as substrate in the perfused pig liver (cf. Figs. 8 and 9).

Infusion of glycerol and glycerate at a concentration of 3 mM did not give rise to any production of pyruvate and lactate. It therefore seems likely that triokinase is responsible for the pyruvate formation from glyceraldehyde under our experimental conditions. However virtually no lactate (or glucose) production was found at glyceraldehyde concentrations corresponding to those observed during steady state fructose elimination.

The major metabolites given off from the liver metabolizing fructose or glyceraldehyde are lactate and glucose. In Fig. 10 the output

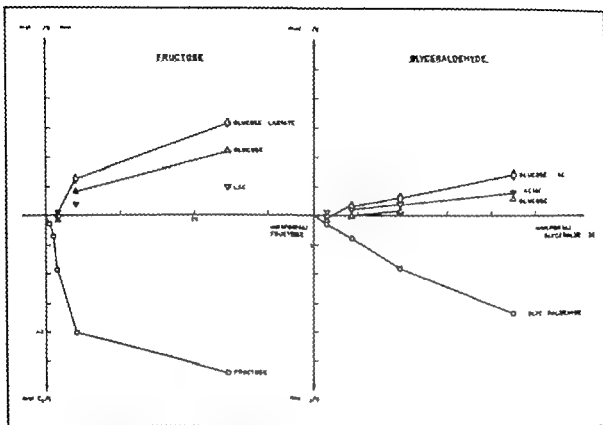


Fig. 10 Output of glucose and L-lactate and uptake of fructose and D-glyceraldehyde respectively calculated as C_2 units in dependence of the concentration of substrate in the affluent medium

of these substances is compared to the uptake of fructose and glyceraldehyde respectively. The glucose formation relative to lactate is much higher with fructose than with glyceraldehyde. With both substrates only about 20 per cent of the substances infused is accounted for as glucose + lactate. This of course means that the remaining part is either metabolized completely to CO_2 and water or deposited in (as glycogen, fat or phosphorylated). Even if it is assumed that the oxygen uptake is used for the catabolism of the added substrate a reasonable estimate of the CO_2 product must be obtained with sufficient quantitative

Acid production. Fig. 11 shows the acid production during experiments with fructose and glyceraldehyde. The titrations are compared with the uptake of the substrate in each of the experimental periods. A simple connection between these two parameters is not to be expected, but some information may be obtained. For instance, in the first period of the fructose experiment the fructose uptake is nearly equal to the hydrogen ion output. The organic acids measured include lactate, pyruvate, glycerate, acetoacetate, β -hydroxybutyrate, citrate, malate, and glutamate. No change in the concentration of ketone bodies was observed, but malate and citrate showed a marked increase (about 3 times). How much total organic acids measured accounted for only 40–50 per cent of the titration suggesting intracellular accumulation of organic acids. In experiments with phosphorylated substrate there is a striking

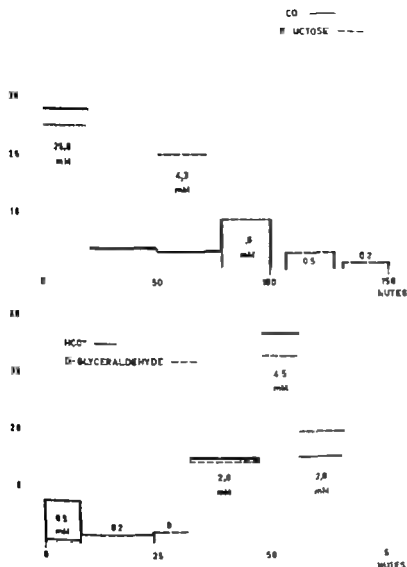
pH 7.4
pH 7.4

Fig. 11 Acid production and elimination of fructose and D-glyceraldehyde respectively. The figures inserted in the columns represent average concentrations in the affluent medium.

similarity in the uptake of substrate and the output of acid equivalents. Again the organic acids (pyruvate, lactate, glycerate) account for only part of the titration (about 30 per cent). The rest seems to be intracellular accumulation of acids.

Metabolic parameters

The oxygen uptake has consistently been observed to increase when fructose is infused

both in man (12) in the cat (7), and in the present experiments. Hassinen (4) however in perfused rat liver found a (transient?) decrease of oxygen uptake. In the pig liver an average increase of 30 per cent was found. At least two factors would tend to increase respiration, *viz.* an increased ADP concentration, which is believed to result from the fast phosphorylation of fructose, and the decreased ATP concentration, which will activate pyruvate dehydrogenase (9) and there-

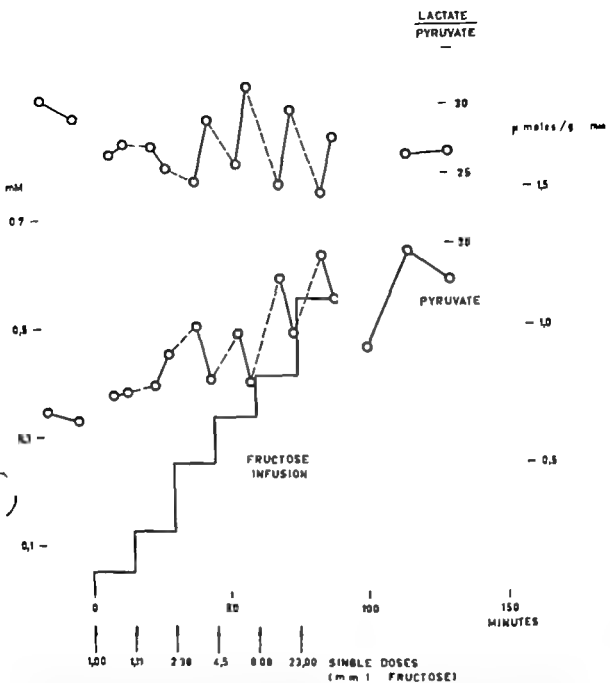


Fig. 12. Changes in lactate-pyruvate ratio during stepwise increasing elimination rate of fructose. Single doses of fructose were administered as indicated, and the concentration levels kept constant with stepwise increasing fructose infusion.

measure the adenosine phosphates in biopsies, were unfortunately unsuccessful.

The cytoplasmic redox state as measured by the lactate/pyruvate ratio showed small but characteristic changes when the fructose load is altered as seen in Fig 12. At each increase in the fructose infusion rate a transient decrease in the ratio was observed caused by an increased pyruvate concentra-

fore increase the input of pyruvate in the TCA cycle as pyruvate is present in abundance under these conditions. Attempts to

tion. The increased pyruvate level may be caused by increased activity of pyruvate kinase, which is known to be activated by fructose-1 phosphate (8). On the other hand the temporary decrease in ATP assumed to accompany fructose phosphorylation would release the inhibition of pyruvate dehydrogenase and thereby cause a fall in the pyruvate concentration. The biphasic curve might be caused by the consecutive action of the two mechanisms. (The changes in lactate/pyruvate ratio are small both with fructose and with glyceraldehyde)

CONCLUSIONS

Fructose is eliminated by the isolated pig liver in a concentration dependent way resembling Michaelis kinetics. The maximal uptake was about 3 μ moles per g liver per min. Half this rate was observed at a concentration about 5 mM (K_m).

The experimental findings are in agreement with a strong regulation of the aldolase reaction, resulting in accumulation of fructose-1-phosphate in the liver

Experiments with glyceraldehyde alone indicate that several processes are involved in the metabolism of this substance. Glycerate and glycerol were identified as intermediates. The relation between concentration and rate of metabolism of glyceraldehyde is a complicated one.

The output of glucose and lactate when fructose or D-glyceraldehyde is infused accounts for about 20 per cent of the elimination, but the production of acid equivalents is considerably higher suggesting intracellular accumulation of acids.

The oxygen uptake during fructose metabolism was increased about 80 per cent.

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THE INTERRELATIONSHIP BETWEEN FRUCTOSE AND ETHANOL METABOLISM IN THE ISOLATED PERFUSED PIG LIVER

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Abstract In the isolated perfused pig liver the rate of ethanol elimination is about $0.7 \mu\text{mol/min/g}$. The acetate produced from ethanol is metabolized at a rate of about $0.5 \mu\text{mol/min/g}$. Metabolism of ethanol does not affect oxygen or acetate uptake in this preparation. Alcohol dehydrogenase activity in pig liver is about $2 \mu\text{mol/min/g}$ and is assumed to account for practically all of the ethanol oxidation observed under the experimental conditions. Fructose increases the ethanol elimination rate to more than twice the control value. Oxygen consumption is similarly increased during this stimulation. A relationship between the fructose elimination rate and the stimulation of ethanol elimination is obtained. Pyruvate and D-glyceraldehyde stimulate ethanol elimination, but in both cases the increase is less than 50% of the increase observed with fructose. Uncoupling of oxidative phosphorylation from respiration by dinitrophenol does not affect the basal ethanol elimination rate, but it decreases the fructose effect on ethanol oxidation to less than half. Accumulation of reduced metabolites during stimulation of ethanol elimination by fructose

and D-glyceraldehyde only accounts for a minor part of this effect. From these observations it is assumed that an energy dependent cyclic process (the 'malic enzyme shuttle') converts the reduction equivalents produced in the alcohol dehydrogenase reaction as NADH to NADPH.

Fructose has been found to accelerate ethanol metabolism in man, in dogs, and in rats (8, 10, 11, 13, 14, 16). In other studies small or no effects have been noted (5).

The interaction of fructose and ethanol metabolism may throw light upon important metabolic regulations in the liver. The isolated perfused pig liver is a suitable experimental model for the study of this problem, because it has greater metabolic resemblances with the human liver than rat liver has, because it is free from the regulatory mechanisms which the body exerts on the liver *in situ* and because it permits experimental manipulations which are not feasible in man.

The team responsible for this investigation further includes H. Koudahl, K. Tønnesen, F. Vølle Hansen and K. Winkler.

CONTINUOUS INFUSION OF ETHANOL

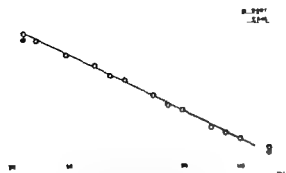


Fig. 1 The concentration of ethanol in afferent (O) and efferent blood (●) during pig liver perfusion with continuous infusion of ethanol (0.45 mmole/min).

The technique of isolated pig liver perfusion has been described in the previous paper together with the fructose metabolism of that preparation (12).

ETHANOL METABOLISM IN THE ISOLATED PIG LIVER

Fig. 1 shows the disappearance of ethanol from the medium following continuous infusion of 0.45 mmole/min of ethanol for 40 minutes. The elimination rate of ethanol is constant from concentrations of about 10 mmole/l to about 1 mmole/l, as reflected by linear fall in concentration and a constant concentration difference between afferent and efferent blood.

Fig. 2 shows the elimination curve after a single injection of 21 mmole of ethanol. There is a small amount of ethanol in the medium before the injection. The concentration of acetate in the medium rises almost linearly during the first period of constant elimination rate of ethanol, and falls after the ethanol has been eliminated. The elimination rate of ethanol is calculated from the slope of the elimination curve, the volume of perfusate, determined by ^{131}I labelled

SINGLE INJECTION OF ETHANOL

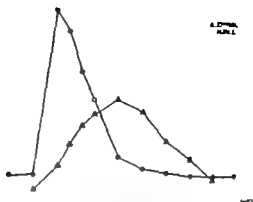


Fig. 2 The elimination of a single dose of ethanol (O) and the production and subsequent elimination of acetate (Δ) in the isolated perfused pig liver

albumin, and the hematocrit. The loss of ethanol from the system by evaporation in the bubble oxygenator was insignificant.

The conversion of acetate by the liver during elimination of ethanol is assessed as the difference between the calculated production of acetate from ethanol and the observed accumulation of acetate in the medium. When no production from ethanol takes place the elimination of acetate is determined as for ethanol.

Table I shows the data for ethanol and acetate elimination in the isolated perfused pig liver. The elimination of ethanol was about one third of the value observed for the human liver *in vivo* (9). About 50 per cent of the acetate produced from ethanol is activated in the isolated liver against less than 25 per cent in the intact human liver. In a liver studied without a simultaneous break-down of ethanol, a similar elimination of acetate was seen. Therefore elimination of ethanol apparently does not inhibit the activation of acetate.

The oxygen uptake of the isolated liver appears to be unaffected by ethanol. About 60 per cent of the oxygen uptake can be accounted for by oxidation of ethanol to acetate. Furthermore the table shows that

Table I. Ethanol metabolism of the isolated perfused pig liver

Period	$\mu\text{mol/min/g} \pm \text{S.E.M. (number of experiments)}$		
	Ethanol	Oxygen	Acetate
No ethanol	—	1.23 ± 0.05 (8)	0.45 ± 0.04 (1)
Ethanol	0.71 ± 0.05 (6)	1.19 ± 0.17 (6)	0.47 ± 0.04 (4)
Supernatant + ethanol	2.14 ± 0.19 (6)		

the activity of alcohol dehydrogenase in the supernatant fraction of pig liver homogenate is considerably greater than the elimination rate in the perfused organ. The K_m of the enzyme for ethanol is about 1.5 mmol/l at pH 8.8.

At an ethanol concentration of 30 mmol/l the ethanol elimination rate was the same. This indicates that no ethanol oxidizing system with a K_m much higher than for alcohol dehydrogenase contributes significantly to the observed ethanol elimination rate. This is supported by experiments with pyrazole known to inhibit alcohol dehydrogenase (Table II). While the inhibition of ethanol elimination is almost complete, acetate elimination is not affected. The lactate to pyruvate ratio of the effluent blood is sharply reduced.

EFFECT OF FRUCTOSE AND FRUCTOSE METABOLITES

The effect of fructose on ethanol elimination is shown in the experiment of Fig 2,

Table II. The effect of pyrazole (8 mmol/l) on ethanol metabolism of the isolated perfused pig liver

Experiment	Period	$\mu\text{mol/min/g}$		Ratios	
		Ethanol	Acetate	I/P	H/A
84	Control	0.77	0.42	340	5
	Pyrazole	0.15	0.42	30	10
90	Control	0.71	0.41	210	12
	Pyrazole	0.00	0.35	17	6

in which a constant infusion of 1.52 mmol/min of ethanol and a stepwise infusion of fructose, 0.00 0.26 0.85, 1.71, and 2.56 mmol/min, was given. The increase in ethanol elimination is reflected by a reduced accumulation of ethanol in the medium, as well as by an increase in the concentration difference between affluent and effluent blood. The effect appears to depend on the dose of fructose given. In Fig 4 the relation between the elimination rate of ethanol and the concentration of fructose in the medium is shown together with the curve of the fructose concentration-elimination relationship. The latter curve is identical with curves obtained in the absence of ethanol (12). With the highest concentration of fructose used, the elimination rate of ethanol is more than doubled, and we therefore can confirm the fructose-ethanol effect in this preparation.

The ability of fructose to stimulate the oxidation of ethanol has been attributed to its rapid conversion to D-glyceraldehyde or pyruvate. Both substances stimulate the oxidation of ethanol (6, 14, 15, 17). It was therefore of interest to compare their effect with that of fructose in the same preparation.

In two experiments (Table III) pyruvate was infused, and concentrations of pyruvate in the medium of 1.3 and 20 mmol/l were obtained. It is seen that despite a fairly high uptake of pyruvate by the liver the stimulation of ethanol elimination was moderate, viz. 30–50 per cent.

In two experiments (Table IV) the effect

CONTINUOUS INFUSION OF ETHANOL
STEPWISE INFUSION OF FRUCTOSE

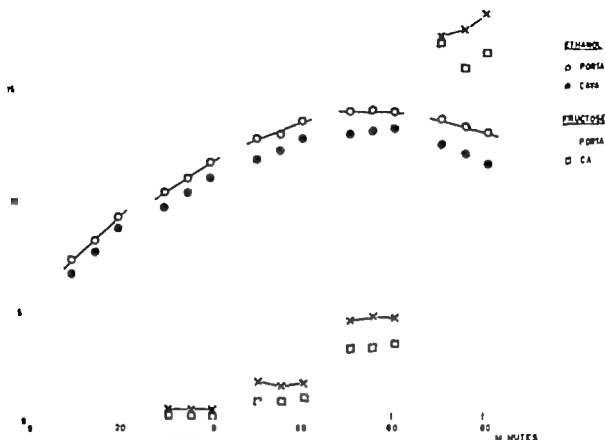


Fig 3 Infusion rates Ethanol 1.52 mmol/min.
Fructose 0.26 0.66 1.71 and 2.86 mmol/min.

of D-glyceraldehyde infusion was studied. The concentration of D-glyceraldehyde in the medium was 2 and 4 mmol/l, respectively. This is four to eight times higher than that observed during fructose stimulation of et

hanol elimination. The ethanol elimination rate is increased, but the stimulation by D-glyceraldehyde is smaller than by fructose although the elimination rates (in μmol/min/g) are similar.

Table III The effect of pyruvate on ethanol metabolism of the isolated perfused pig liver

Experiment	Period	Pyruvate		Ethanol elimination rate μmol/litre	L/P (effluent blood)
		mean conc. mmol/litre	elimination rate μmol/min/g		
103	Control	0.02	0.00	1.00	751
	Pyruvate	1.3	2.0	1.48	87
111	Control	0.03	0.00	1.09	125
	Pyruvate	20.0	3.5	1.29	0.48

FRUCTOSE EFFECT ON ETHANOL ELIMINATION IN ISOLATED PERFUSED PIG LIVER

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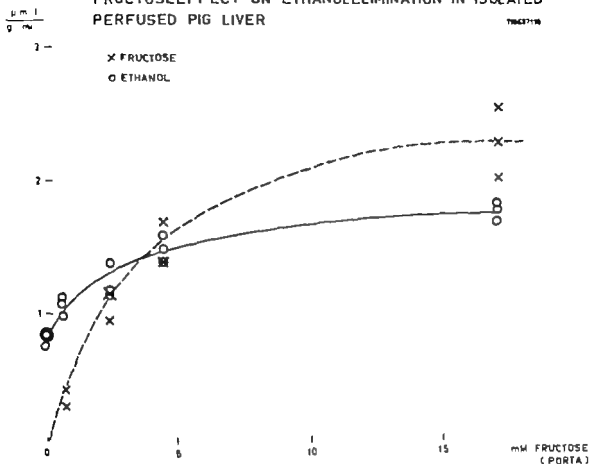


Fig. 4. The data are derived from the same experiment as shown in Fig. 3.

PRODUCTION OF REDUCED METABOLITES DURING THE FRUCTOSE EFFECT

The rate of ethanol oxidation is considered to be determined by the rate of reoxidation of

cytoplasmic NADH formed in the alcohol dehydrogenase catalyzed reaction, and not by the alcohol dehydrogenase activity itself (2, 3) Stimulation of ethanol oxidation may therefore be the result of an increased re-

Table IV The effect of D-glyceraldehyde on ethanol metabolism of the isolated perfused pig liver

Experiment	Period	D-glyceraldehyde mean conc. mmoles/ litre	elimination rate μmol/ min/g	Ethanol elimination rate μmol/ min/g	L/P (effluent blood)
107	Control	0.1	0.07	0.50	402
—	D-GALD	2.0	1.43	0.79	410
113	Control	0.03	0.00	1.33	336
—	D-GALD	4.0	2.67	1.63	400

CONTINUOUS INFUSION OF ETHANOL AND FRUCTOSE

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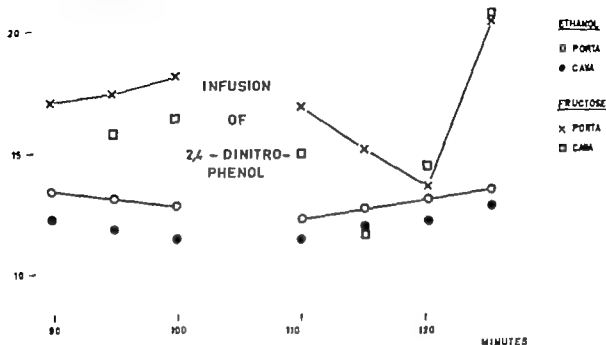


Fig 6 Infusion rates Ethanol 1.58 mmoles/min. Fructose 2.56 mmoles/min. 2.5 mmoles of 2,4 dinitrophenol was infused in the interval from 100 to 110 minutes.

effects suggest that 60 per cent of fructose stimulation of ethanol oxidation depends on the presence of intramitochondrial ATP since DNP action will deprive the mitochondrial matrix before the cytosol of the high energy phosphate.

In rat liver the stimulation of ethanol oxidation by D-glyceraldehyde has been observed (14). If the mechanism is a mere reduction of D-glyceraldehyde to glycerol this

effect should be independent of ATP (4, 7). However addition of rotenone abolished the stimulation completely although oxygen consumption in both controls and stimulated assays were only inhibited 50 per cent (15). In isolated hepatocytes pyruvate stimulation of ethanol oxidation was also completely inhibited by rotenone (1).

MALIC ENZYME SHUTTLE

The common need for mitochondrial ATP by these three effects (pyruvate, D-glyceraldehyde, and fructose) indicates a common stimulatory mechanism. Speculations about this

Table VII. The stimulation of ethanol elimination by fructose and the effect of 2,4-dinitrophenol on this stimulation

Period	Ethanol	$\mu\text{mol/min/g}$ Oxygen	Fructose	Ratio L/P	H/A
Control	0.8	0.8	0.0	414	18
Fructose	1.8	1.8	2.5	843	28
Fructose + DNP	1.2	2.2	3.9	189	22

MALIC ENZYME SHUTTLE

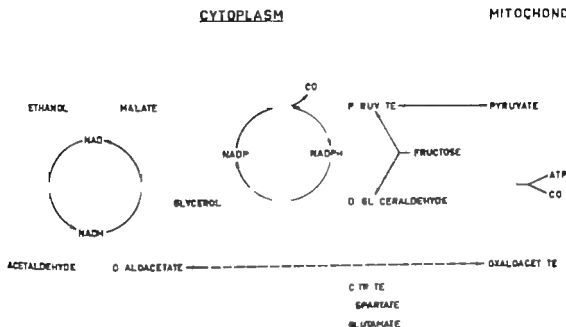


Fig. 7 The reactions shown are thought to be responsible for the energy dependent part of the fructose induced stimulation of ethanol elimination.

mechanism have led to the proposal of what may be named the malic enzyme shuttle (15) outlined in Fig. 7. At least four enzyme activities are necessary. Alcohol dehydrogenase oxidizes ethanol to acetaldehyde, and this process is probably limited by the increase in the NADH to NAD⁺ ratio. The malate dehydrogenase reaction produces NAD⁺ from NADH in the cytosol from oxaloacetate. The malate formed in this reaction can be converted to pyruvate by malic enzyme if NADP⁺ is present. Pyruvate is carboxylated intramitochondrially by an energy requiring process and oxaloacetate is formed. Transport of oxaloacetate from the mitochondria to the cytosol is an indirect process, but can proceed via citrate if malate

is present or as aspartate if glutamate is present.

The carboxylation of pyruvate depends on the pyruvate concentration in the physiological concentration range (K_m about 0.4 mM). Therefore pyruvate will stimulate the activity of the shuttle.

This shuttle actually converts NADH to NADPH and would soon stop if NADP⁺ was not reformed from NADPH. An obvious way to do this is to form fatty acids. The acetyl-S-CoA which is necessary for these processes is formed intramitochondrially from acetate or pyruvate and can be transported to cytoplasm together with oxaloacetate in the form of citrate. Also other processes in the liver utilize NADPH so it can be assumed that besides fatty acid synthesis there is a basal turnover.

D-glyceraldehyde stimulation of 'malic enzyme shuttle can now be explained as an increase in NADP⁺ formation from NADPH

as a result of D-glyceraldehyde reduction to glycerol by the NADP dependent alcohol dehydrogenase. Fructose stimulation of this cycle is attributed to its very rapid conversion to D-glyceraldehyde and pyruvate which stimulates the malic enzyme shuttle in two different ways and therefore possesses more stimulatory power than either of its two metabolites.

If this explanation of the fructose effect is correct, then a considerable part of the fructose effect on dehydrogenation of ethanol does not lead to an increased mitochondrial oxidation of the NADH produced, but to anabolic processes via the malic enzyme shuttle involving storage of the reduction equivalents in the form of fat or other reduced substances. This model in contrast to earlier explanations of the fructose effect includes the $\text{NADP}^+ \rightarrow \text{NADPH}$ redox system which is known to change during ethanol metabolism (18) even though the NADP dependent alcohol dehydrogenase does not oxidize ethanol. Furthermore the requirement for mitochondrial ATP is explained by this model.

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MODIFICATION OF METABOLIC EFFECTS OF ETHANOL BY FRUCTOSE

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Abstract. Present knowledge of the metabolic interactions between fructose and ethanol in man and laboratory animals is reviewed. The possible biochemical mechanisms of these interactions are considered. Some new data on the effects of fructose and ethanol on carbohydrate and lipid metabolism are included.

Fructose increases the effects of ethanol on the hepatic lactate/pyruvate and α -glycerophosphate/dihydroxyacetone phosphate ratios. Thus it augments the basic metabolic effect of ethanol, i.e. reduction of the hepatic redox state.

The ethanol-induced increase in hepatic α -glycerophosphate concentration has been regarded as an important factor in the pathogenesis of the acute alcoholic fatty liver. But although fructose causes hypertriglyceridaemia and augments the effect of ethanol on hepatic α -glycerophosphate concentration, it was found to diminish the ethanol induced accumulation of triglycerides in the liver. Glucose had similar effect. The reason for this is uncertain, but the inhibition of peripheral lipolysis by fructose and glucose might be partly responsible.

The most important effect of ethanol on carbohydrate metabolism is to inhibit hepatic gluconeogenesis by producing NADH in excess. Although the production of glucose from fructose is not directly dependent on the redox state of the liver it is slightly inhibited by ethanol *in*

vitro. However *in vivo* fructose is effectively converted to glucose even during ethanol oxidation, and in ethanol induced hypoglycaemia it rapidly restores the blood glucose level.

Fructose is one of the few compounds reported to accelerate the elimination of ethanol at least in some metabolic states (12, 47, 51, 53, 54). Much effort has been expended in endeavours to elucidate the mechanism of this effect. Because these studies throw some light on the metabolic effects of ethanol, we have a reasonable amount of data about the metabolic interactions of fructose and ethanol in man and laboratory animals. These interactions have been studied especially by Lundquist *et al.* (23-37, 53, 54), by Papenberg *et al.* (46) and by Hassinen *et al.* (18, 20, 57, 58).

The aim of this paper is to survey the effects of fructose on ethanol induced changes in the hepatic redox state and on the alterations in carbohydrate and lipid metabolism occurring during ethanol oxidation.

EFFECTS OF FRUCTOSE AND ETHANOL ON THE REDOX STATE OF THE LIVER NAD/NADH COUPLE

We do not fully understand the mechanism which regulates the rate of ethanol metabolism. However the ethanol-induced accumulation of reducing equivalents in the liver has generally been regarded as an important factor at least *in vivo* (24, 50). Many workers have investigated the effect of fructose on the ethanol-induced reduction of the hepatic redox state in the hope of throwing further light on the way in which fructose increases the rate of elimination of ethanol.

Fructose itself alters the equilibrium of the different redox couples in the liver to a more reduced state, as indicated by the increase of the hepatic lactate/pyruvate (L/P), malate/oxaloacetate (MAL/OAA) and α -glycerophosphate/dihydroxyacetone phosphate (α -GP/DAP) concentration ratios (5, 22, 48). Surface fluorometric measurements on the perfused rat liver also show that NADH fluorescence increases transiently after addition of fructose (19). A possible mechanism for the fructose-induced redox changes is discussed by Hassinen et al. in this same symposium (21).

Data about the effect of fructose on the ethanol-induced reduction of the hepatic NAD/NADH ratio are somewhat contradictory. In 1956 Holzer and Schneider suggested that fructose might accelerate the rate of ethanol oxidation by functioning as a hydrogen acceptor (25). They had no experimental data to support this hypothesis. In experiments on liver slices, Thieden and Lundquist found that fructose really does reduce the ethanol induced increase in the hepatic L/P ratio (33). However the L/P ratios in their control experiments were abnormally high (about 50). In experiments on perfused livers Papenberg et al. (46) found that fructose significantly enhanced the ethanol induced redox changes, as indicated by the

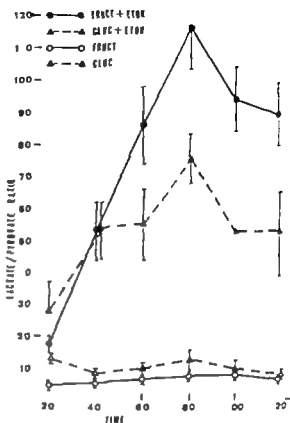


Fig. 1. Lactate/pyruvate concentration ratio (L/P) of the perfusion medium after addition of different substrates. The livers of normal fasted rats were perfused with 100 ml of Krebs-Ringer bicarbonate solution, pH 7.4, temperature 35°C, for 20 minutes without any substrate. 1 mmole of fructose or glucose with or without 2 mmole of ethanol was then added. Each point represents the mean \pm SEM of the values from 6 to 8 perfusions of different livers.

hepatic L/P and α -GP/DAP ratios. Ylikahri et al. (58) found that the L/P ratio in the perfusion medium was significantly higher when livers were perfused with fructose and ethanol than when they were perfused with glucose and ethanol (Fig. 1). The results of *in vivo* experiments are qualitatively similar. When fructose and ethanol were administered intravenously to anaesthetized rats the hepatic α -GP/DAP ratio was increased significantly more than with ethanol alone (45). The same fact was observed when fructose

Table I. Production or elimination of metabolites in liver perfusion during the first hour after addition of substrates

Livers were perfused with Krebs-Ringer bicarbonate solution as described earlier (35). After 30 minutes of perfusion 10 mM glucose or 10 mM fructose with or without 20 mM ethanol was added to the perfusion medium. Results are expressed as $\mu\text{moles}/100 \text{ g body wt}/\text{hour}$. Values are means \pm SEM. Number of experiments in parentheses.

Substrate added	Metabolite produced (+) eliminated (—)			
	Glucose	Fructose	Lactate	Pyruvate
Glucose	—	—	—	—
Glucose + ethanol	-60.8 ± 13.9 (5) -60.8 ± 9.7 (5)	—	$+14.0 \pm 1.4$ (5) $+29.4 \pm 7.1$ (5)	$+1.6 \pm 0.9$ (5) $+0.5 \pm 0.1$ (5)
Fructose	$+100 \pm 19$ (7)	-291 ± 10 (5)	$+101 \pm 6$ (5)	$+13.9 \pm 1.8$ (5)
Fructose + ethanol	$+124 \pm 9$ (12)	-259 ± 34 (6)	$+116 \pm 12$ (7)	$+1.1 \pm 0.2$ (7)

(or glucose) was given to rats by stomach tube at the same time as ethanol (Fig. 5). Thus fructose seems to augment the ethanol induced reduction of the redox state of the hepatic NAD/NADH couple.

The reduction of the hepatic NAD/NADH ratio is the most important metabolic effect of ethanol (13, 28, 32). Almost all the metabolic disturbances produced by ethanol have been linked to this redox change (13, 28, 32). Now it appears possible that when fructose affects this basic change, it also alters other metabolic effects of ethanol, for instance ethanol induced changes in the metabolism of carbohydrates and lipids. These points will be discussed in the latter part of this paper.

FRUCTOSE AND ETHANOL-INDUCED CHANGES IN HEPATIC CARBON DIOXIDE PRODUCTION

The normal liver rapidly converts ethanol to acetate, which is transported to other tissues and there oxidized (see ref. 13). The oxidation of ingested ethanol accounts for more than 70 per cent of the energy requirements of the liver (see ref. 13). Hepatic oxygen consumption is unchanged, which means that ethanol monopolizes hepatic energy metabolism. Hepatic CO_2 production is inhibited and the respiratory quotient de-

creases to very low values (15, 55). This means that ethanol inhibits the function of the citric acid cycle. The basic biochemical mechanism of this effect is unsolved, but one important factor is probably the ethanol-induced accumulation of NADH in the liver (13, 55). In support of this hypothesis Lindros (23) found a negative correlation between the L/P ratio and the respiratory quotient in perfused liver.

Fructose is also rapidly metabolized in the liver (Table I) (49, 58) and affects the ethanol induced changes in the hepatic redox state, but we could find no reliable information on how fructose affects the ethanol induced changes in liver CO_2 production. Fructose alone has been reported to increase CO_2 production of the liver (54). In human experiments ethanol with fructose depressed hepatic CO_2 production even more than ethanol alone (54). Forsander and Himberg found that in high concentrations glucose inhibited the ethanol-induced decrease in the hepatic respiratory quotient (14), but they did not test fructose.

FRUCTOSE AND ETHANOL-INDUCED CHANGES IN CARBOHYDRATE METABOLISM

Ethanol is known to affect the blood glucose level (4, 38, 52). The direction of the

change depends on the metabolic state of the subject (see references 13 and 38). These effects are also considered by Hillborn et al. in this Symposium (23). In fasted subjects ethanol causes hypoglycaemia owing to inhibition of hepatic gluconeogenesis by excess NADH (11 16 36). In fed subjects ethanol increases the blood glucose concentration (32), owing to ethanol induced glycogenolysis mediated by a neuro-humoral mechanism (see ref. 38).

In the normal liver fructose is rapidly phosphorylated to fructose-1-phosphate and metabolized via glycolysis much faster than glucose, as indicated by increased lactate production (Table I) (49 58). A considerable part of fructose is converted to glucose. Gluconeogenesis from fructose, unlike that from pyruvate and alanine, is not directly dependent on the redox state of the liver because there is no dehydrogenation step in this pathway (29).

Reports on the combined effects of fructose and ethanol on liver carbohydrate metabolism are contradictory. Madison et al. (39) found that ethanol did not inhibit the conversion of fructose to glucose and that fructose effectively raised the blood glucose level during alcohol-induced hypoglycaemia, although glutamate and α -ketoglutarate had no effect. In perfused livers Exton and Park found that gluconeogenesis from fructose was not significantly inhibited by ethanol (10). Krebs et al. used perfused livers, too, but found that ethanol markedly inhibited production of glucose from fructose (29). Our results confirm this (Table I) (38). Papenberg et al. (46) also found that in perfused rat liver ethanol inhibited uptake of fructose and output of glucose. The inhibition found by several authors may be due to the fact that during ethanol oxidation the DAP formed from fructose-1 phosphate in a reaction catalyzed by aldolase II is rapidly converted to its reduced form, α -GP which is used for triglyceride synthesis (37).

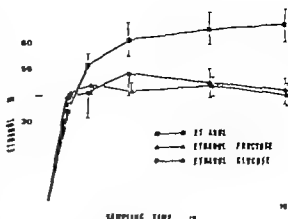


Fig. 2. Blood ethanol concentrations after administration of ethanol with glucose or fructose. Ethanol (8.0 g/kg body wt) alone or with glucose (8.7 g/kg body wt) or fructose (8.7 g/kg body wt) was administered to normal fed rats by stomach tube. Blood samples were taken from the tip of the tail. Each point represents mean \pm SEM of the values from 8 rats.

In the intact organism the interactions of fructose and ethanol are even more complex than in the isolated liver. Tygstrup et al. have performed elegant human experiments to elucidate this question (54). They found that the splanchnic output of glucose was three times as great during ethanol fructose infusion as during fructose infusion alone. Thus ethanol seemed to increase the conversion of fructose to glucose in the human organism.

We have recently made some experiments on fed rats to study the interactions of fructose and ethanol in intact animals. The rats were divided into five groups. Group I received glucose 8.7 g/kg body wt (isocaloric to 50 g of ethanol) by stomach tube. Group II received the same amount of fructose and group III ethanol 50 g/kg body wt. Groups IV and V received glucose 8.7 g + ethanol 50 g/kg body wt and fructose 8.7 g + ethanol 50 g/kg body wt. Blood samples for ethanol, fructose and glucose determinations were taken from the tip of the tail. Determinations were carried out by the methods which we have used earlier (37 38).

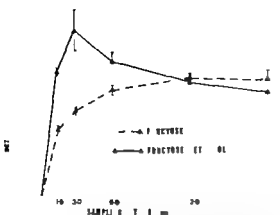


Fig 3. Blood fructose concentrations after administration of fructose with or without ethanol. Experimental conditions as in Fig. 2. Each point represents mean \pm SEM of the values from 8 rats.

Blood ethanol concentrations were significantly higher in group III than in groups IV and V (Fig. 2). But the values 15 minutes after administration were still similar in all groups. This suggests that the differences found during the latter part of the experiment are not attributable to delayed absorption of ethanol in groups IV and V but to the acceleration of ethanol elimination by fructose and glucose.

Fructose concentrations in peripheral blood were low all the time (Fig. 3). This is probably due to the rather slow absorption of fructose and its rapid uptake by the liver. During the first hour after administration the concentrations were higher in the fructose + ethanol group than in the fructose group. We cannot as yet offer any explanation for this phenomenon.

Blood glucose concentrations were highest in group IV (glucose + ethanol) (Fig. 4). This indicates that glucose combined with ethanol increases blood glucose much more than glucose alone. Ethanol hardly affects glucose absorption, but it causes glycogenolysis in the liver and thus raises the blood glucose level. Ethanol has also been reported to inhibit peripheral utilization of glucose (34).

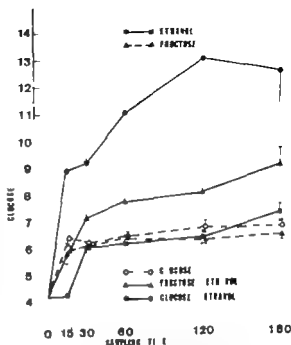


Fig 4. Effect of oral administration of fructose, glucose and ethanol on blood glucose concentration in rats. Experimental conditions as in Fig. 2. Each point represents mean \pm SEM of the values from 8 rats.

This may account for the high blood glucose concentrations in all groups of experimental animals which had received ethanol.

Fructose alone increased the blood glucose concentration (Fig. 4). Ethanol augmented this effect, indicating that fructose is rapidly converted to glucose even during ethanol metabolism.

It can be concluded that ethanol may slightly inhibit the production of glucose from fructose *in vitro* but that *in vivo* fructose rapidly abolishes the hypoglycaemia induced by ethanol.

EFFECT OF FRUCTOSE ON ACUTE ETHANOL-INDUCED FATTY LIVER

The mechanism by which one large dose of ethanol induces the accumulation of tri

glycerides in the liver has been studied intensively. Some investigators attribute the phenomenon to the effect of ethanol on peripheral lipolysis or to direct toxic effects of ethanol on the liver or to both these mechanisms (2, 6, 8, 9, 26). Others hold that ethanol *per se* does not produce fatty liver but that the changed metabolic conditions in the liver during the oxidation of ethanol favour triglyceride synthesis (27, 41, 42, 56). The increased formation of triglycerides is assumed to be linked with the increased liver NADH content. The reduction of NAD to NADH favours the reduction of DAP to α -GP which has been considered as an important regulator of hepatic lipogenesis (17, 40–42, 56). Studies on rats treated with pyrazole, which effectively inhibits the oxidation of ethanol, have indicated that ethanol oxidation is necessary for the induction of acute alcoholic fatty liver (1, 7, 40). Results from thyroxine-treated and clofibrate-treated rats also suggest that ethanol-induced reduction of the hepatic redox state is essential in the pathogenesis of acute alcoholic fatty liver (3, 30, 31, 56). Thyroxine and clofibrate prevent the ethanol-induced redox change, and they also inhibit the production of fatty liver by ethanol (30, 31, 56).

Several reports show that fructose, even alone but especially with ethanol, increases the hepatic content of α -GP and so stimulates triglyceride synthesis (5, 41, 42, 56, 59). Moreover fructose has been found to induce triglyceridaemia in man and laboratory animals (43, 44). Thus it might be expected that fructose would worsen the alcoholic fatty liver.

To clarify this question we studied the effect of fructose on ethanol induced hepatic triglyceride accumulation. Five groups of rats were treated as described on page 000 (I glucose, II fructose, III ethanol, IV glucose + ethanol, V fructose + ethanol). The animals were anaesthetized 4 or 12 hours after

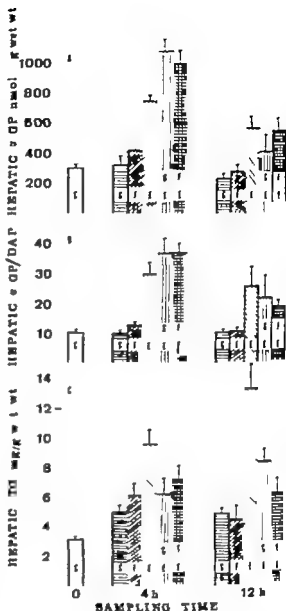


Fig. 5 Effects of fructose, glucose and ethanol on hepatic α -glycerophosphate (α -GP) concentration, hepatic α -glycerophosphate/dihydroxyacetone phosphate (α -GP/DAP) ratio and hepatic triglyceride (TG) concentration in rats. Rats were treated as in Fig. 2. Liver samples were taken under light ether anaesthesia and placed in liquid nitrogen. Symbols: C = control (no treatment), E = treated with glucose, F = treated with fructose, E = treated with ethanol, G + E = treated with glucose + ethanol, F + E = treated with fructose + ethanol. Each bar represents mean \pm SEM of the values from 5 to 8 rats.

the treatment, the abdomen was opened and a piece of liver was removed to liquid nitrogen for α -GP, DAP and triglyceride determinations. Immediately afterwards the abdominal aorta was cut and blood collected for determination of serum triglycerides and free fatty acids. The methods used have been described earlier (57-58).

As seen in Fig 5A, ethanol caused a significant rise in hepatic α -GP concentration at 4 hours. Both fructose and glucose augmented this effect significantly. Twelve hours after the treatment α -GP concentrations were still increased in the ethanol groups, but no additive effect of fructose was observed.

The curves for the α -GP/DAP ratios were parallel to those of α -GP (Fig 5B) because the DAP concentration was almost unchanged during the experiment. Thus during acute intoxication both fructose and glucose augmented the redox effect of ethanol.

Both fructose and glucose increased the hepatic triglyceride content (Fig 5C) but the effect of ethanol was significantly greater. It was somewhat surprising to find that in both the glucose + ethanol and fructose + ethanol groups the hepatic triglycerides rose far less than in the ethanol group, although the α -GP concentration was higher in the two first-mentioned groups. In all groups which had received ethanol the changes in serum triglyceride concentrations were about the same (Fig 6A). Serum free fatty acid concentration was increased by ethanol but decreased by ethanol + glucose and ethanol + fructose (Fig. 6B). This decrease is probably attributable to inhibition of peripheral lipolysis by high serum glucose concentrations.

The present results show that although the reduction of the hepatic redox state is one of the essential changes underlying the production of fatty liver by ethanol, it is not the only important factor. These ex-

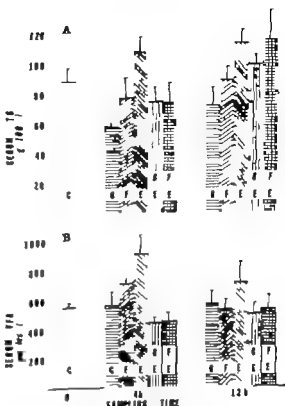


Fig 6 Effects of glucose, fructose and ethanol on serum triglycerides (TG) and free fatty acid (FFA) concentrations in fed rats. Rats were treated as in Fig. 2. Blood samples were taken under light ether anaesthesia by cutting the abdominal aorta. Each bar represents mean \pm SEM of the values from 6 to 8 rats.

periments do not throw light on the mechanism by which fructose and glucose inhibit the production of fatty liver by ethanol. Inhibition of peripheral lipolysis is certainly one factor but probably not the only one. Domanski et al. have also found that glucose is protective against the production of fatty liver by ethanol, but they offer no explanation for this effect (7).

The doses of fructose and glucose used in these experiments were large. A more detailed study including dose-response relationships, is needed to solve the problem of how fructose and glucose prevent ethanol-induced fatty liver.

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EFFECTS OF FRUCTOSE, GLUCOSE AND GLYCERALDEHYDE ON THE TOXICITY OF ETHANOL IN HYPERTHYROID RATS

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Abstract. The effects of single or repeated intraperitoneal injections of fructose and glyceraldehyde were compared with those of glucose and saline in the treatment and prevention of the toxicity of ethanol in hyperthyroid rats. Glyceraldehyde potentiated the lethal effect of ethanol on these animals. The dose of ethanol (3 g/kg body wt.) produced only slight ketosis. Fructose, despite of its antiketogenic action, was equal in value to glucose or saline in the treatment of the intoxication syndrome. Ethanol induced hypoglycemia, which was previously assumed to be causally related to the toxicity of ethanol in hyperthyroid rats, was counteracted by giving repeated intraperitoneal injections of fructose or glucose, but the mortality of the animals was the same as in saline-treated controls. The results suggest that hypoglycemia is not the principal cause of death in ethanol-intoxicated hyperthyroid rats.

It has been shown that ethanol may especially after fasting (5) or in the hyperthyroid state (1), induce severe hypoglycemia which sometimes leads to coma or even death. A rapid reversal of this condition can be achieved with injections of glucose, at least in the case of ethanol-induced hypoglycemia

of the type developing after fasting and poor nutrition (7, 16). This treatment has not been tried in hyperthyroid patients developing hypoglycemia after ethanol. In experiments with hyperthyroid rats Ylikahri (20) has shown that large doses of ethanol produce severe hypoglycemia soon after administration and we have previously demonstrated that ethanol also induces slight ketosis in these animals (11) although generally described as an antiketogenic agent. The role of these effects in the toxicity of ethanol in the hyperthyroid state is not established.

In order to test the possibility of a causal relationship between the ethanol-induced hypoglycemia or ketosis and the toxicity of ethanol we studied the effects of single and repeated intraperitoneal injections of fructose, glucose and glyceraldehyde on ethanol induced mortality in hyperthyroid rats. Fructose was compared with glucose because of its glucogenic and antiketogenic properties and because it has been reported to stimulate the rate of ethanol oxidation in liver tissue (2, 17). In liver perfusion experiments it has also been demonstrated

that gluconeogenesis from fructose is not inhibited by ethanol in the hyperthyroid state (21)

MATERIAL AND METHODS

Fed male Wistar rats, four months of age and weighing from 150 to 250 g were used in the experiments. All the animals were given an ordinary laboratory diet (containing 34 / of calories as protein) and tap water *ad libitum*. The animals were rendered hyperthyroid by injecting 0.2 mg/kg body weight of 3,3',5-triiodo-L-thyronine (T₃) in slightly alkaline solution intraperitoneally on seven successive days. The experiments were performed on the eighth day.

In the first part of the study thirty two rats were given 3 g/kg body weight of ethanol in intraperitoneally in saline (20 / w/v solution). Four of them died within half an hour and the remaining twenty-eight animals were randomly divided into four groups of seven rats and immediately given glyceraldehyde (1 g/kg body wt.) fructose (1 g/kg body wt.), glucose (1 g/kg body wt.) or saline (equal volume). Levels of blood glucose and ketone bodies (β -OH butyrate + acetoacetate + acetone) were determined before giving ethanol and one half, one and two hours, after its administration. The injections of glyceraldehyde, fructose, glucose and saline were given intraperitoneally just after the blood sample taken half an hour after the administration of ethanol. The time course of mortality of the animals was recorded and values of blood glucose and total ketone bodies were measured by methods described elsewhere (4, 19).

In a second series of eighteen rats, divided into three groups we studied the effects of repeated injections of fructose, glucose or saline

on the mortality of hyperthyroid rats. The first group of animals was given a single intraperitoneal injection of a 20 / (w/v) ethanol (3 g/kg body wt.) solution in saline. The other two groups received the same amount of ethanol together with fructose or glucose (1 g/kg body wt.). Thereafter five intraperitoneal injections of fructose (1 g/kg body wt.), glucose (1 g/kg body wt.) or saline (equal volume) were given hourly. Blood glucose and ethanol values were determined on the surviving rats six hours after the injection of ethanol. Blood samples were taken from the tip of the tail and blood alcohol was determined by gas chromatograph as described earlier (6).

RESULTS AND DISCUSSION

The results indicate (Table I) that the dose of ethanol used in these experiments was lethal when given intraperitoneally to hyperthyroid rats, thus confirming the previous report of Ylikahri (20). In euthyroid rats the LD₅₀ for intraperitoneal injection of a 10 or 20 % (w/v) solution in saline is given as 3 g/kg (18).

A single intraperitoneal injection of 3 g glyceraldehyde solution was found to potentiate the toxicity of ethanol in hyperthyroid rats. All the animals in this group died within one and a half hours after the injection but most of the animals given glucose fructose or saline survived for more than three hours. When the same dose of glyceraldehyde was given to four hyperthyroid animals without ethanol none of them died. The last blood samples taken

Table I Effect of a single intraperitoneal injection of glyceraldehyde fructose glucose or saline on the ethanol induced mortality of hyperthyroid rats

Treatment	Number of animals	Died within 1/2-3 hr	Died within 3-6 hr	Survived
Glyceraldehyde (1 g/kg body wt.)	7	7	—	—
Fructose (1 g/kg body wt.)	7	2	5	—
Glucose (1 g/kg body wt.)	7	2	5	—
Saline (equal volume)	7	2	5	—

Thirty-two fed male Wistar rats were given ethanol (3 g/kg body wt.) intraperitoneally in saline and four of them died within half an hour. The remaining twenty-eight animals were randomly divided into four groups of seven rats and immediately given glyceraldehyde fructose, glucose or saline.

Table II. Blood glucose levels in hyperthyroid rats after injection of 3 g/kg ethanol intraperitoneally

Group	Number of animals	Blood glucose	
		Before ethanol injection	Last value before death
Surviving more than 3 hr	15	71 \pm 0.6	74 \pm 1.6
Surviving less than 3 hr	12	68 \pm 0.6	47 \pm 1.1

The animals are the same as those described in Table I. The figures represent the mean \pm S.D. Blood glucose values are expressed as nmol/ml of blood.

Table III. Blood ketone body levels in hyperthyroid rats after injection of 3 g/kg ethanol intraperitoneally

Group	Number of animals	Blood ketone bodies	
		Before ethanol injection	Last value before death
Surviving more than 3 hr	15	164 \pm 47	231 \pm 65
Surviving less than 3 hr	12	167 \pm 65	273 \pm 95

The animals are the same as those described in Table I. The figures represent the mean \pm S.D. Values of total ketone bodies (β -OH-butyrate + acetoacetate + acetone) are expressed as nmol/ml of blood.

from the glyceraldehyde-treated animals given ethanol revealed slight hypoglycemia in every case, but similar blood glucose values were also obtained from animals treated with fructose, glucose or saline, half an hour before death ensued. The values recorded from the total material treated with single injections indicated that the fatal outcome after ethanol was in fact preceded by a significantly ($p < 0.001$) lowered level of blood glucose as can be seen in Table II. However it must be pointed out that in most animals the lowered blood glucose level prevailed for only a short period before death. A single injection of fructose or glucose did not prolong the life of the animals, as compared with the saline-treated controls, although a transient rise in blood glucose was noted after their administration.

An examination of the development of blood ketone bodies in those animals which survived for more than three hours and those which survived for less than three

hours indicated an equal and significant ($p < 0.01$) increase in both groups as can be seen in Table III. However this increase was rather slight and the level of ketone bodies was not influenced to any great extent by the single injections of fructose, glucose or glyceraldehyde. In any case it can be concluded that the fatal outcome after ethanol is not connected with severe ketosis in the hyperthyroid state.

Heim and his coworkers have reported that the lethal dose of ethanol is doubled in euthyroid cats when 4 g/kg body wt. of fructose is given before the experiment (8, 9). The mechanism of this effect is unresolved. It is generally accepted that fructose can accelerate the rate of elimination of ethanol in the body (3, 12, 14, 15). However fructose has no marked effect unless it is present in the blood throughout the degradation of ethanol, and therefore must be used in large amounts.

In order to see if a large amount of



FRUCTOSE AND DIABETES

EFFECT OF FRUCTOSE AND OTHER SUGARS ON ISLET FUNCTION IN VITRO

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Abstract. Glucose and mannose stimulate insulin release, insulin biosynthesis and calcium uptake in rat isolated islets. Their insulinotropic action is enhanced by caffeine or theophylline. Ribose and xylitol might also exert a minor insulinotropic action. Fructose and galactose do not stimulate insulin release whether in the presence or absence of methylxanthine (100 mM) and do not affect insulin biosynthesis or calcium uptake by the islets. These converging findings on several parameters of islet function support the concept that the insulinotropic effect of sugars is dependent on their intracellular metabolism, or possibly on the activation of highly-selective glucoreceptor

venous administration of fructose in man (1, 9, 31), dog (16) and rabbit (35). The direct insulinotropic action of fructose might be more marked in fetal pancreas and during childhood. Thus, in the preparation of cultured fetal rat pancreas, a significant insulinotropic action of fructose was noticed in the presence of caffeine (20). A transient stimulation of insulin release by fructose was seen in newborn pigs (43) and sheep fetuses (8), although not in newborn calves (6). In normal children or young patients with Type 1 glycogenosis, fructose injection might also provoke an early secretory response (17, 33). This insulinotropic action of fructose is absent in fructose intolerance (3, 7, 33). Taken as a whole, these findings suggest that (i) the direct insulinotropic action of fructose is negligible in adults and possibly more marked during childhood and (ii) the modest insulinotropic effect of fructose is dependent on the accumulation of a glycolytic metabolite below the fructose-1 phosphate-aldolase level. In more general terms, the above-mentioned findings are com-

The effect of fructose on insulin release has been the subject of a number of investigations. Except in one study (42), little or no direct insulinotropic action of fructose was found in vitro with either pieces of rabbit pancreas (4), pieces of rat pancreas (28), rat isolated islets (19) and the perfused rat pancreas (13, 41). Also in vivo small to insignificant changes in the level of circulating insulin were seen after oral or intra-

patible with the concept that the insulinotropic action of sugars is secondary to their metabolism in the beta cell, a process conceivably influenced by developmental and/or dietary factors, as well as by hereditary metabolic defects.

This concept has been recently challenged because (i) insulin release and changes in the level of intermediates and cofactors in the beta cell might occur independently and (ii) sugars which are not metabolized (e.g. galactose) or which are even potential inhibitors of glucose metabolism (e.g. glucosamine) were found to stimulate insulin release under certain experimental conditions (21-23). In the light of these recent observations, we have reexamined the influence of various sugars, including fructose, upon insulin secretion and other parameters of islet function *in vitro*.

MATERIAL AND METHODS

All experiments were performed with either pieces of pancreas or isolated islets removed from fed albino rats, and incubated for 90 min at 37°C in bicarbonate buffered media containing albumin (3.0 mg/ml). The methods used for the measurement of insulin release from either pieces (28) or islets (27, 30), and insulin biosynthesis (27) and calcium uptake (30) by isolated islets have been described in detail elsewhere.

RESULTS

Effects of individual sugars on insulin release

Glucose stimulates insulin release in both pieces of pancreas (Fig. 1) and isolated islets (28, 30). The relationship between the glucose concentration of the incubation medium and the rate of insulin secretion is characterized by a sigmoidal curve with a threshold value for the insulinotropic action of glucose at a glucose level close to 5.0 mM. The maximal rate of secretion is achieved

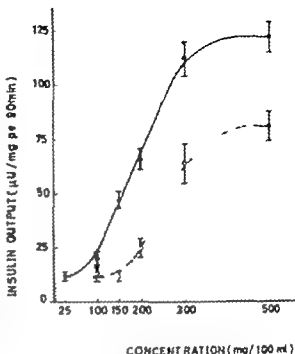


Fig. 1. Mean values (\pm SEM) for insulin output by pieces of pancreas incubated at various glucose (closed circles, solid line) or mannose (open circles, dotted line) concentrations.

at concentrations ranging between 18.7 and 27.8 mM (28, 30).

Mannose also stimulates insulin secretion in both pieces of pancreas (Fig. 1) and isolated islets (25, 29). The effect of mannose is less marked than that of glucose at all concentrations (Fig. 1).

In the present system, none of the following sugars or polyols, when used at a 16.7 mM concentration, significantly increases the basal level of insulin release: galactose, fructose, ribose (23), sorbitol (unpublished observations), xylitol and ribitol (23).

Combined effects of glucose and other sugars or polyols on insulin release

In the presence of a low glucose concentration (1.7 to 2.8 mM) and at high concentration (15.0 or 16.7 mM) fructose, galactose

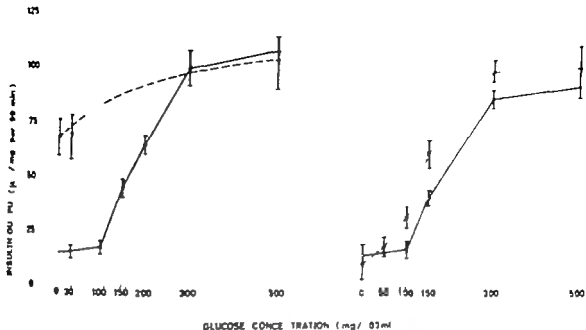


Fig. 2. The mean control values (\pm SEM) for insulin output by pieces of pancreas incubated at various glucose concentrations are shown as open circles (solid line). The mean changes (\pm SEM) in secretion rate induced by either mannose (300 mg/100 ml on the left) or xylitol (233 mg/100 ml on the right) were added to substracted from their respective control values and are shown as closed circles (dotted line). Overlapping SEM refer to insignificant changes.

ribose, xylose, arabinose, 2-deoxyglucose, ribitol and xylitol also fail to significantly modify insulin release (23, 28).

At intermediate glucose concentrations (3.6 to 16.7 mM), namely in a range of glucose values where small increments in glucose concentration cause marked increases in insulin output, ribose and xylitol (16.7 mM) significantly but modestly increase glucose-induced insulin secretion (23). The effects of other sugars, under these experimental conditions, has not yet been tested in the present system.

At maximal glucose concentration (16.7 to 27.8 mM), none of the carbohydrates so far examined (i.e. mannose, ribose, xylitol and ribitol, all 16.7 mM) has any significant effect upon insulin secretion (23, 25).

As outlined in greater detail elsewhere (24) these data indicate that some carbohydrates (mannose, ribose, xylitol) mimic the effect of glucose and, by doing so, cause a shift to the left of the sigmoidal curve illustrated in Fig. 1. When the shift is of sufficient amplitude (e.g. with mannose) a significant stimulation of insulin release is observed in the absence of glucose (Fig. 2 left). Otherwise (e.g. with ribose and xylitol) a significant insulinotropic effect is only observed at intermediate glucose concentrations (Fig. 2 right).

Effects of sugars on calcium uptake and insulin biosynthesis

Glucose increases the net uptake of calcium in islets incubated in the presence of 45 calcium (30). Mannose also provokes an accumulation of 45 calcium in the islets whereas both galactose and mannoheptulose fail to significantly affect the basal calcium uptake by the isolated islets (29). These findings are consistent with the hypothesis that the insulinotropic action of various sugars is mediated through an accumulation of calcium in the beta cell (29).

Glucose, and to a lesser extent mannose stimulate insulin biosynthesis by isolated islets. Xylitol, fructose and galactose have no obvious effect upon insulin biosynthesis (36). These findings confirm previous observations (22).

Combined effects of sugars and methylxanthines on insulin release

Theophylline (14 mM) and caffeine (1.3 mM) potentiate glucose-induced insulin secretion (2, 26). In both pieces of pancreas and isolated islets, no significant effect of theophylline is seen at low glucose concentrations (0 to 2.8 mM). At higher glucose levels (5.6 to 16.7 mM), theophylline markedly enhances glucose-induced insulin release the increment in secretion rate being most marked at the highest glucose concentration (2, 26). The enhancing action of theophylline increases as its concentration in the incubation medium is raised from 0.3 to 14 mM (26).

Theophylline (14 mM) also potentiates mannose-induced insulin secretion. Again, the increment in secretion rate is much more marked at high than at low mannose concentration (25).

In more recent experiments, we have studied the effect of a higher concentration of methylxanthine (100 mM) upon sugar induced insulin release in isolated islets. As expected, the methylxanthines significantly enhanced insulin release in the presence of either glucose or mannose (16.7 mM). In contrast, in the absence of any sugar or in the presence of either galactose or fructose (16.7 mM) the addition of theophylline or caffeine (100 mM) only caused minor and usually insignificant increases in insulin output (unpublished observations).

Methylxanthines are thought to increase insulin output by causing a translocation of calcium within the beta cell from an organelle-bound pool into the cytosol (2). In order for such translocation to cause sus-

tained insulin release, the beta cell has to be simultaneously exposed to some other insulinotropic agent itself preventing the outward transport of the translocated calcium across the membrane of the beta cell (2). Therefore, it is not surprising that sugars which fail to provoke calcium accumulation in the beta cell also fail to support the insulinotropic action of methylxanthines.

DISCUSSION

In the present experiments, various parameters of beta cell function were influenced in parallel fashion by each of the different sugars. Thus, glucose and to a lesser extent mannose, stimulated insulin release, calcium uptake and insulin biosynthesis by the incubated islets. These sugars also allowed theophylline or caffeine to exert their insulinotropic action. In contrast, we could not detect any obvious effect of either galactose or fructose upon these various biochemical parameters. In our view these converging findings on several parameters reinforce the concept that the effect of sugars upon the beta cell function is dependent on the activation of a glucoreceptor with selective affinity for a few hexoses or more likely on the intracellular metabolism of these particular sugars.

If insulin release is due to the accumulation of a metabolic signal rather than to the activation of a poorly selective glucoreceptor (21) it might be expected that the pancreatic response to various sugars is influenced by constitutional and environmental factors including species and age, which might modify the enzymatic pattern of the beta cell. And, indeed, the effect of certain sugars upon insulin release appears to be affected by such factors.

We have already discussed the possible developmental influence on fructose-induced insulin release. Also if there is general agreement on the failure of galactose to

directly stimulate insulin release (4 10 11 17 19 23, 31 35 37 42), the insulinotropic action of ribose and pentitols appears to be markedly influenced by a series of experimental conditions. For instance xylitol markedly stimulates insulin release in dog (15 16, 18, 44) whereas the insulinotropic action of this pentitol is small if not debatable in man (8, 39) monkey (44) and rat (20, 22, 34). Ribose might also stimulate insulin release in dog (11 14) and rabbit (35 38, 42), its effect being small and transient in man (12, 40).

In summary the present study clearly indicates that various carbohydrates, including epimers of glucose, vastly differ in their ability to stimulate insulin release and biosynthesis in the beta cell.

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THE CORTICOID INSULIN AND GROWTH HORMONE RESPONSES TO INTRAVENOUS FRUCTOSE IN MEN AND WOMEN

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Abstract. The corticoid, insulin and growth hormone (HGH) responses to intravenous fructose were studied in 20 men and 12 healthy oophorectomized women, who were given an infusion of 100 mg/kg fructose over a period of five minutes. Blood samples were obtained before the infusion and at intervals for one hour thereafter.

Significant rises in plasma corticoid concentration were not observed, but significant falls appeared at one hour in all subjects. Plasma insulin concentrations rose significantly within 15 minutes of the infusion and fell to sub-fasting values by one hour in the males, but similar changes were not found in the females. Fasting HGH concentrations were significantly higher in the females than in the males and 1 one hour after fructose these values fell in the former and rose in the latter to a significant degree.

In the men the changes in plasma insulin were directly related to the changes in plasma glucose. Changes in plasma corticoid and HGH concentrations were inversely related to the changes in plasma insulin.

In the women the above interrelationships were not demonstrable, but both the 30 minute changes in plasma glucose and insulin were found to be directly related to the degree of obesity.

These responses to fructose in the men are similar to those induced by glucagon and it is

suggested that the initial response to fructose may be the release of glucagon.

Some controversy has existed about the efficacy of fructose as a stimulus to insulin release. Kilo et al. (7) demonstrated insulin secretion in anaesthetised dogs who were given an unspecified quantity of fructose. Grodsky et al. (8) obtained only slight stimulation of insulin release in isolated rat pancreas in the presence of a fructose concentration of 500 mg/100 ml, whereas Coore and Randle (3) were unable to demonstrate any insulin secretion from isolated rabbit pancreas using fructose concentrations of 300 mg/100 ml. In 1969 we reported significant rises in plasma insulin concentration in healthy men after giving 31 Gm fructose intravenously over a period of five minutes (1), and later we described similar changes in a comparable group of men following acute myocardial infarction (2). In both these instances plasma fructose concentrations did not exceed 120 mg/100 ml.

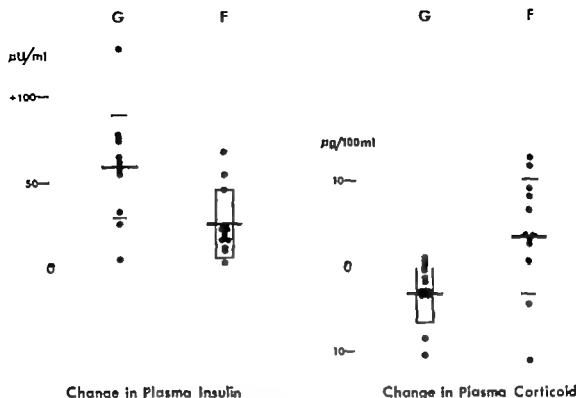


Fig 1 Change in plasma insulin and corticoid concentrations 30 minutes after giving either 31 g glucose (G) or fructose (F) intravenously in 12 healthy men. Means \pm 1 SD given.

In the normal subjects studied there was in addition a significant rise in plasma corticoid concentrations and in view of the frequent occurrence of abdominal pain after such large doses of fructose it seemed possible that the insulin response might have resulted from a reaction to stress and not as a direct response to fructose itself (Fig 1). This suspicion was supported by the finding that the insulin response and the rise in blood glucose after fructose were both directly related to the rise in plasma corticoid (Figs. 2 and 3).

In the present study we have examined the insulin, corticoid and growth hormone (GH) responses to a much smaller intravenous bolus of fructose, which was found not to cause any physical discomfort whatever.

MATERIAL AND METHODS

Twenty men with normal glucose tolerance (some of whom were convalescent hospital in-patients, the others being healthy medical personnel) and twelve healthy middle-aged oophorectomized women (all of whom were outpatients) were studied. All subjects readily volunteered to participate in this study after the details of the test had been explained to them. Following a 10–12 hour overnight fast and abstinence from smoking, each subject was given an infusion of 20 per cent fructose intravenously in a dose of 100 mg/kg over a period of five minutes. All outpatients rested recumbent for at least 18 minutes prior to the test. In 11 subjects blood samples were obtained fasting and at 30 and 60 minutes after the start of the infusion. In eight men additional samples were obtained at 10 and 20 minutes. None of the volunteers experienced either abdominal or chest discomfort during or after the fructose infusion.

Blood samples were analysed for plasma glucose by glucose oxidase procedure (9) using a standard Boehringer kit, and plasma corticoid was measured by fluorimetry (10), insulin and GH were measured by radioimmunoassay the former using a Sephadex bound insulin antibody

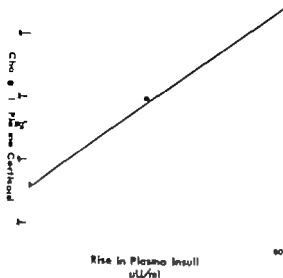


Fig 2 Relation between 30 minute insulin and corticoid change after 31 g fructose i.v in 12 healthy men ($r = 0.63$, $t = 2.90$ $p < 0.02$)

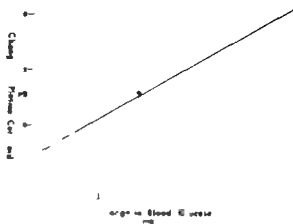


Fig 3 Rel tio between 30 minut glucose and corticoid chang after 31 g fructose v in 12 healthy men ($r = 0.59$ $t = 2.29$ $p < 0.05$)

(13) and the latter by a modification of the standard antibody/antigen precipitation method (6). In the women obesity was assessed by measuring body weight and height and from this the Fat Score was derived, where

$$\text{Fat Score} = \frac{3}{\text{height (cm)}} \sqrt{\text{weight (kg)}} \times 1000$$

RESULTS

The baseline data on the subjects studied is given in Table I. The details of the response of these subjects to intravenous fructose are shown in Table II.

Plasma glucose concentrations rose significantly within 10 minutes of the infusion by a mean of 13 mg/100 ml ($t = 6.40$ $p < 0.001$), and remained significantly elevated until 30 minutes after the infusion, but by one hour the plasma glucose concentration was no longer significantly above fasting values. Similar changes were not seen in the women.

Mean plasma corticoid concentrations re-

mained unchanged to the first 30 minutes in the males, but by one hour there was a significant mean fall of 3.4 µg/100 ml ($t = 2.77$ $P < 0.01$). In the females the mean plasma corticoid concentrations had already

Table I Basal values of subjects studied. Means \pm SEM given

	Men (20)	Women (12)
Age (range)	43 (24-65)	52 (47-57)
Weight kg	73 (± 2.3)	64 (± 2.9)
Fat Score	—	25 (± 0.5)
Plasma glucose mg/100 ml	83 (± 2.2)	85 (± 1.4)
Plasma corticoid µg/100 ml	31 (± 1.2)	18 (± 1.6)
Plasma insulin µU/ml	18 (± 1.8)	10 (± 1.9)
Plasma growth hormone µU/ml	2 (± 0.6)	8 (± 1.2)

Table II. Response to i.v fructose 100 mg/kg in men and women. Means above fasting values given \pm SEM Significant differences from fasting values given thus

		Change from fasting values at			
		10 min	20 min	30 min	60 min
Plasma glucose mg/100 ml	Men	+12.9 ** (\pm 2.0)	+8.9 **** (\pm 1.6)	+8.5 **** (\pm 1.2)	+17 (\pm 1.8)
	Women	—	—	-0.7 (\pm 2.3)	+0.1 (\pm 1.2)
Plasma corticoid μ g/100 ml	Men	+14 (\pm 1.3)	+1.0 (\pm 1.6)	+0.4 (\pm 1.2)	-3.4 ** (\pm 1.2)
	Women	—	—	-3.1 ** (\pm 1.0)	-4.8 *** (\pm 0.9)
Plasma insulin μ U/ml	Men	+11.9 ** (\pm 2.0)	+5.9 ** (\pm 2.0)	+0.3 (\pm 1.0)	-3.3 (\pm 1.4)
	Women	—	—	+0.4 (\pm 0.9)	+0.4 (\pm 0.6)
Plasma growth hormone μ U/ml	Men	+0.1 (\pm 0.1)	+0.8 (\pm 0.4)	-0.1 (\pm 0.5)	+1.3 † (\pm 0.7)
	Women	—	—	-0.7 (\pm 1.2)	-1.3 † (\pm 1.0)

p < 0.05

p < 0.02

** p < 0.01

**** p < 0.001

† p < 0.05

fallen significantly by 30 minutes ($t = 3.22$, $p < 0.01$) and fell still further at one hour by a mean of 4.8 μ g/100 ml ($t = 5.20$ $p < 0.001$)

In the men, plasma insulin concentrations rose by a mean of 12 μ U/ml at 10 minutes, but by 30 minutes these had fallen to fasting values and at one hour the mean plasma insulin concentration was significantly below the fasting value by 3.3 μ U/ml ($t = 2.32$, $p < 0.05$). In the women in whom measurements were only made at 30 and 60 minutes, no significant mean changes in plasma insulin concentration were found.

Mean fasting plasma GHG concentrations were significantly higher in the women at 8 μ U/ml as compared with the men in whom the value was 2 μ U/ml ($t = 4.61$ $p < 0.001$). In the latter there was a slight fall in the GHG concentrations at 30 minutes and thus

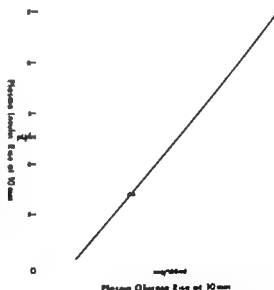


Fig 4 Relation between 10 minute insulin and glucose change after 100 mg/kg fructose i.v in 8 healthy men $y = 1.23x - 3.92$ ($r = 0.86$, $t = 4.10$ $p < 0.01$)

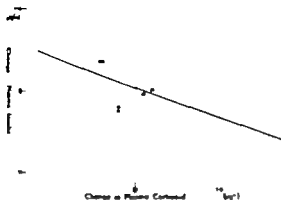


Fig 5 Relation between 30 minute insulin and corticoid change after 100 mg/kg fructose i.v. in 28 healthy men, $y = -0.38x + 0.38$ ($r = -0.46$ $t = 2.18$, $p < 0.05$).

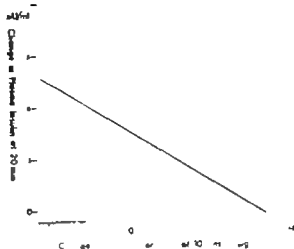


Fig 6 Relation between 10 minute insulin and 10 minute corticoid change after 100 mg/kg fructose in 8 healthy men $y = -1.22x + 7.81$ ($r = -0.82$ $t = 3.45$ $p < 0.01$).

was followed by a small rise at one hour whereas there was a small progressive fall at both 30 and 60 minutes in the women. The mean change in HGH concentration at one hour was $+1.3 \mu\text{U/ml}$ in the men and $-1.3 \mu\text{U/ml}$ in the women ($t = 2.30$ $p < 0.05$).

On comparing the plasma insulin and glucose changes at 10 minutes after the infusion in the males (Fig 4), a significant direct correlation was found ($r = 0.86$, $t = 4.10$ $p < 0.01$). No correlation was found between the plasma corticoid change and the corresponding change in plasma glucose at any time after the fructose infusion. The 30 minute plasma insulin and corticoid changes (Fig 5) were inversely related ($r = -0.46$, $t = 2.18$, $p < 0.05$). Similarly on comparing the 10 minute corticoid change with the 20 minute insulin change (Fig 6) a much more significant inverse correlation was found ($r = 0.82$, $t = 3.45$ $p < 0.02$). Analogous comparisons between these parameters were not found in the female subjects, but the changes observed in this group were discovered to be related to the degree of obesity. The Fat Score, which is the same as the reciprocal of the Ponderal Index (in c.g.s.

Fat Score

30—

28

26—

24

22

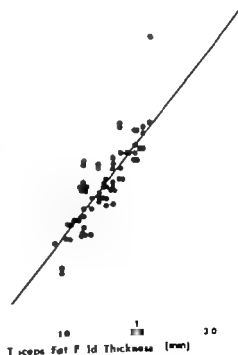


Fig 7 Relation between Fat Score and Triceps Fat Fold Thickness in 104 middle-aged women $y = 0.23x + 20.68$ ($r = 0.82$, $t = 14.35$ $p < 0.0001$).

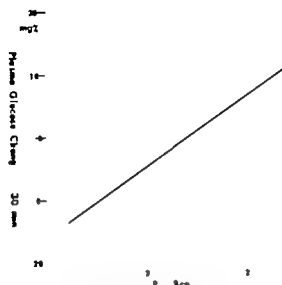


Fig 8 Relation between 30 minute plasma glucose change after 100 mg/kg fructose i.v. and Fat Score in 12 healthy middle-aged women $y = 2.87x - 73.49$ ($r = 0.63$, $t = 2.59$ $p < 0.05$)

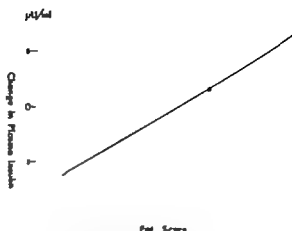


Fig 9 Relation between 30 minute plasma insulin change after 100 mg/kg fructose i.v. and Fat Score in 12 healthy middle-aged women $y = 1.44x - 37.31$ ($r = 0.75$, $t = 3.61$ $p < 0.01$)

units) correlates well with the Triceps Fat Fold Thickness (in mm) in middle-aged women (Fig 7). When the Fat Score was compared with the change in plasma glucose at 30 minutes (Fig 8) a significant correlation was found ($r = 0.63$ $t = 2.59$ $p < 0.05$). Similarly the change in plasma insulin at 30 minutes was directly related to the Fat Score (Fig 9) to a significant degree ($r = 0.75$ $t = 3.61$ $p < 0.01$).

A significant inverse correlation was found between the insulin and HGH changes at 30 and 60 minutes (Fig 10) in the males ($r = 0.57$ $t = 3.96$ $p < 0.001$). A similar relationship was not found in the females.

DISCUSSION

The present study confirms beyond doubt that even relatively small intravenous doses of fructose in man are followed by significant rises in plasma insulin concentration. The temporal relationship of insulin secretion after fructose infusion is similar to that

seen after intravenous glucose in so far as in both instances the maximal rises occur within about 10 minutes of infusion. Although the true plasma glucose concentrations rose *pari passu* with the rises in plasma insulin and the magnitude of these responses were significantly correlated, this does not necessarily imply that the rise in plasma glucose *per se* caused the associated insulin secretion. Had the glucose rise caused the insulin secretion one might have expected to see a better correlation between the earliest glucose rise and the subsequent rise in plasma insulin concentration, but such a relationship was not found.

Stimulation of the pituitary-adrenal axis by the fructose infusion was not demonstrable in this study and in fact plasma corticoid concentrations fell in the same way and to the same extent as have been demonstrated previously after glucose infusions (1). Hence in this instance stress could not be implicated as the mechanism responsible for either the rises in plasma glucose or plasma insulin concentrations.

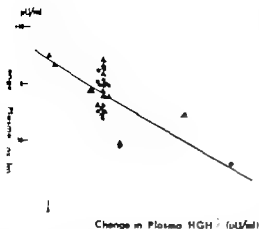


Fig. 10. Relation between 30 minute (open triangles) and 90 minute (closed circles) plasma insulin and HGH changes after 100 mg/kg fructose L. in 20 healthy men $y = -1.15x - 1.48$ ($r = -0.57$, $t = 3.90$, $p < 0.001$).

The lack of significant mean changes in plasma insulin concentrations after fructose infusion in the women studied was not surprising in view of the negligible changes at 30 minutes in the men. In the absence of blood samples taken at 10 and 20 minutes after infusion it should not be assumed that a similar pattern of insulin secretion after fructose occurred in the women. In view of the small range of insulin and glucose changes at 30 minutes in these subjects it could be argued that this merely represented randomly scattered values about zero. Had this been true one would not have expected to find any relationship between the size or degree of obesity of the women studied and the associated changes in plasma insulin and glucose concentrations after fructose infusion. Since changes in both plasma glucose and insulin were directly related to the degree of obesity it seems likely that these plasma changes were not random variations but reflected a specific response to the fructose infused. It was interesting that the most obese women experienced the greatest glucose and insulin responses. If the magnitude of the glucose and insulin responses to fructose were dose related, as was sug-

gested by Kilo et al. (8) one would have expected the heavier subjects to have shown the largest rises. Hence the relationship between obesity and insulin response might have been an artefact since the most obese women tended also to be the heaviest and hence received most fructose. In this context no correlation was found between the insulin change at 30 minutes and body weight in either the women or the men studied. It should be remembered that since obese subjects have relatively less extracellular fluid (ECF) they would tend to have somewhat higher ECF fructose concentrations than lean subjects in the immediate post infusion period. Alternatively the heightened response to fructose found in the obese women could be the result of either an inherent metabolic difference peculiar to obesity or the release of a metabolite of fructose derived from its catabolism in adipose tissue. Both pyruvate and glycerol, which are intermediaries in the catabolism of fructose could have played a part in augmenting the insulin response to the small changes in plasma glucose (3, 12). This aspect of the insulin response to fructose requires further investigation.

The differences in fasting HGH concentrations found between the males and females were similar to those reported by Frantz and Rabkin (4). In view of the similarity between the action of glucose and the present small dose of fructose in lowering plasma corticoid, it might have been expected that the usual HGH lowering effect of glucose would have been observed in this study after fructose. Whereas fructose infusion caused an initial mean fall in plasma HGH concentration in both groups, this was followed by a rise in the males at one hour — the response being significantly different from that seen in the females in whom the fall continued. Although these changes were small, the pattern was similar to that found after the s.c. injection of glucagon in men (11).

All the changes in the males reported in this study could be explained on the basis of an initial release of glucagon by the fructose infusion, but clearly this requires confirmation.

ACKNOWLEDGEMENTS

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EFFECT OF SUCROSE FEEDING ON GLUCOSE TOLERANCE

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Abstract Feeding high sucrose diets to human volunteers and experimental animals results in impaired glucose tolerance, hyperlipidemia and impaired serum insulin-like activity. Breeding selected rats with impaired glucose tolerance caused by sucrose feeding produces in succeeding generations diabetes, renal arteriosclerosis, and renal and retinal diabetic like angiopathy. The starch fed siblings develop none of these changes. The interaction between genetics and sucrose consumption in the production of diabetes mellitus is thus proved.

This experimental model explains the development of diabetes in Yemenite Jews immigrating to Israel. In the Yemen, where their diet contained no sucrose, the incidence of diabetes was extremely low. After settling in Israel, where they consumed large amounts of sucrose, the incidence of diabetes has risen.

It was suggested by Claude Bernard in 1877 (2) that the organism's tolerance to carbohydrates is affected by previous nutritional factors. Himsaworth (16) has elaborated on this and has shown that a high carbohydrate diet may improve glucose tolerance in normal persons. Uram et al. (24) suggested a hypoglycemic substance in starch.

The extreme change that has occurred in several Jewish ethnic groups as a result of immigrating to Israel, in their external environment, mode and habits of life and food habits offered the possibility to study the effect of these changes on the prevalence of diabetes.

In a field survey on the prevalence of diabetes in Israel, 16,000 people were examined. It was found that the prevalence of diabetes was 1 per cent for the Sephardi ethnic group and 2.5 per cent for the Ashkenazi ethnic group, with an average of 1.8 per cent for the total population (4). However the difference between the Ashkenazi and Sephardi groups was not significant. This incidence of diabetes among Jews in Israel is not greater than that among non-Jews in other parts of the world (1, 15, 17, 18, 21, 23, 25).

On examining about 5000 Yemenite new immigrants to Israel, we could find only 3 cases of diabetes, i.e. the prevalence was almost zero. However on examining Yemenites who settled in the country for more than 25 years, the incidence of diabetes was

Table 1 Food intake per capita per day

Groups	N f males	C kcal	Protein		Fat		Carbohydrates	
			Intak (g)	Animal & margarine	Oil	Total (g)	Sucrose (g)	
Yemenites in Y. men	70	37 + 26	60 ± 3.6	42 ± 3	14 ± 3	351 ± 15	48 ± 1	(1.18)
Yemeni id settlers	70	559 - 118	46 ± 4.4	51 ± 3	30 ± 3	377 ± 31	63 ± 6	(0.9)
Europe n id settlers	29	2618 + f f	60 ± 4	5 ± 4	33 ± 4	353 ± 32	50 ± 2	(12.5)

Y. lives are me \pm SD

F. figures in parentheses = f total calories from sucrose

higher than that observed in the Ashkenazi Western Jew. The same observation was repeated also among the Kurdish new immigrant Jews where no diabetes was found, while among the Kurdish old settlers, the incidence of diabetes was also higher than among the Ashkenazi Jews (4).

A dietary survey carried out with Dr Bavly and Mrs Poznanski among the Yemenites revealed that 1) in the Yemen most of the fat consumed was from animal sources and 2) almost no sucrose was consumed, while in Israel a high percentage of the carbohydrate intake was on the form of sucrose and a large percentage of the fat was in the form of unsaturated fat (Table 1) (7). The possible association of this change in diet, i.e. the increased sucrose consumption, and the increased incidence of diabetes was suggested by us in 1961 (5). Similar observations were made in 1963 in and around Durban, relating the increased incidence of diabetes in Asians and Africans with the increased sucrose consumption (3).

Accordingly in collaboration with Mrs. Teitelbaum two groups of albino rats were put on fully-supplied synthetic diets (Table II) in which the carbohydrate (72 per cent) consisted in the experimental group of sucrose and in the control group of corn starch. After keeping the animals for two months on these diets, oral glucose tolerance was found to be impaired in the sucrose-fed group. Similarly the insulin like activity of the sucrose-fed group was lower and the growth curve was found to be impaired when compared with that of the starch-fed group (Table III) (10). Reducing the protein content in the diet made these changes more severe (11). However the nitrogen balance in the sucrose-fed rats was not different from that of the starch fed rats (12).

In order to rule out the possibility that the impairment of the glucose tolerance was due to a change in the rate of glucose absorption from the gastrointestinal tract.

Table II. Contents of diets (synthetic)

	Percentage by weight		
	Starch	Sucrose	Glucose-Fructose
Vitamin-free casein	18	18	18
Butter	5	5	5
Starch	72	0	0
Sucrose	0	72	0
Glucose	0	0	36
Fructose	0	0	36
Salt and vitamins**	5	5	5

* USP salt mixture no. II.

** Containing 0.2 mg thiamin, 0.2 mg riboflavin, 0.1 mg pyridoxine, 1.6 mg calcium pantothenate, 5 mg niacin, and 100 mg choline chloride per 100 g. Fat soluble vitamins A and D added twice weekly

Intravenous glucose tolerance test was performed. The glucose removal rate (K) was calculated according to the equation.

$$K = \frac{2.3 (\log G_{t_1} - G_{t_2})}{t_2 - t_1}$$

where G_t is the excess blood glucose concentration at time t and is equal to the blood glucose at time t minus the blood

glucose fasting level. The value K represents the percentage removal of the excess glucose per minute

The glucose removal rate (K) from the blood (Table III) was significantly smaller in the sucrose-fed animals (0.03 ± 0.0022) than in the starch fed controls (0.040 ± 0.0028)

In order to clarify whether the impaired glucose tolerance is due to the sucrose or to the glucose and fructose fractions group of albino rats were fed the same synthetic diet but the carbohydrate was supplied as 36 per cent fructose and 36 per cent glucose. The oral glucose tolerance performed after 4 months feeding revealed that it is impaired as much as that of the sucrose-fed animals (Table III)

Togther with Groen (13) human volunteers were fed in alternate 5-weekly periods on diets composed of saturated fat, 40 grams proteins, 70–80 grams carbohydrate, 250–300 grams . . . On changing the kind of carbohydrate in each period from sucrose to bread and vice versa we have shown that after the bread diet period the glucose tolerance test was lower than after the sucrose diet period. The blood cholesterol showed a rise on the sucrose diet and a drop on the bread diet.

Table III. Effect of diet on weight gain, blood glucose and insulin like activity of serum in rats

Diet	No. of animals	Gain in body wt (g)	Blood glucose mg%					Insulin-like activity	
			60'	120'	p ^b	K	p ^b	mg glucose used/100 mg diaphragm	p ^b
Starch	10	80 ± 4.7	120 ± 2.4	110 ± 2.8	—	0.040 ± 0.0028	—	0.750 ± 0.042	—
Sucrose	9	66 ± 11.8	150 ± 4.2	155 ± 3.5	0.05	0.030 ± 0.0022	0.03	0.411 ± 0.071	0.01
Glucose + Fructose	10	78 ± 6.3	154 ± 5.1	162 ± 4.5	0.05	—	—	—	—

Values are means ± SE.

* After being fasted 18 hours, then given glucose load (350 mg/100 g body wt)

^b Probability of difference between the respective groups and the starch group.

See text.

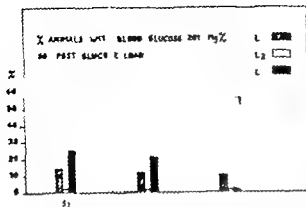


Fig 1 Blood glucose values at 60 minutes following an intragastric glucose load (350 mg/100 g body weight) in the selected generations (S₁, S₂, S₃) of the upward selected lines L₁ and L₂ and the downward selected line L₃.

In collaboration with Mrs Salternik (14) 28 male and 11 female albino rats from the animal house — parent generation (P) — at the age of 21 days, were fed on sucrose diet for two months. At the end of this period, a glucose tolerance test was performed. A male and two females with the highest blood glucose values were mated — Upward Selection — and the descendants of these couples called Lines I and III. Likewise a male and a female from the P generation with the lowest blood glucose values were mated — Downward Selection — and the offspring called Line II. The descendants of Lines I, II and III were also fed on sucrose diet for two months and similarly selected, Lines I and III upwards and Line II downwards. Fig. 1 shows the percentage of animals following an oral glucose load with blood glucose above 200 mg/100 ml in the different selected generations of Lines I, III and II. It shows definitely that with further selection in Lines I and III (upward selection) the percentage of animals with blood glucose above 200 mg/100 ml increases while in Line II (downward selection) there is no difference in the succeeding selected generations.

The rats fed high sucrose diet for long periods developed renal changes of the na-



Fig 2 Starch — fed rat. Normal glomerulus. Hematoxylin and eosin, x120.

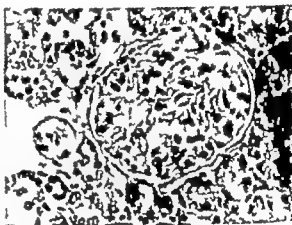


Fig 3 Sucrose — fed rat. The glomerulus is enlarged and there is marked widening of the mesangial areas, hematoxylin and eosin, x120.

ture of diffuse glomerulosclerosis (Figs. 2 and 3) and in electron microscopy a thickening of the basement membrane-like material was observed. (Fig. 4) (8, 20). The trypsin digested retina showed loss of mural and endothelial cells, irregularity of capillary diameter, varicose capillary loops, strand formation and microaneurysms (Figs. 5 and 6) (8).

These lesions in the kidneys and retina are identical with vascular lesions observed in spontaneous and experimentally induced



Fig. 4. Sucrose — fed rat. Electron microscopy of the glomerular mesangium. Note the increase of the mesangial matrix, the electron density of which is identical with the peripheral basement membrane. $\times 22,800$

diabetes. Renal and retinal vascular changes were not observed in any of the starch-fed age-matched controls.

Preliminary studies in *Acomys cahirinus* revealed that sucrose feeding resulted in similar changes as noted in the albino rat, i.e. impaired glucose tolerance, excessive rise in serum cholesterol and triglycerides and an increased enzymatic activity in the liver of enzymes involved in lipogenesis, glycolysis and gluconeogenesis.

It appears that the decisive factor for the glycemic response is the rate of glucose inflow into the circulation and stimulation of the insulin system. Normal rats fed stock

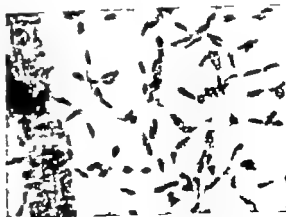


Fig. 5. Starch — fed rat. Digested flat retina (control) showing general normal appearance of the vessels. P.A.S. and hematoxylin, $\times 420$.

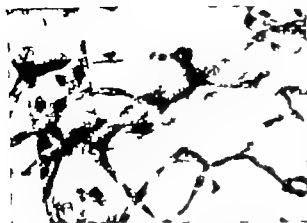


Fig 6 Sucrose — fed rat. Digested flat retina showing diminution of mural and endothelial cells and microaneurysm. P.A.S. and hematoxylin, x420.

diet (Table IV) had a significantly higher blood glucose level response to intragastric sucrose load than to a starch load (0.5 g/100 g body weight) (10). Thus compared to starch, the absorption of glucose from the gastrointestinal tract in the case of sucrose feeding is rapid, leading to a steep post prandial rise in blood glucose with resulting stronger stimulation of the insulin system (22).

These and other experiments (19–24) show that high sucrose diet impairs the carbohydrate balance in both man and animals, as compared to animals fed dietary carbo-

hydrates in the form of starch, while keeping constant in other dietary ingredients. Furthermore, vascular complications characteristic of diabetes have been found in animals fed sucrose.

The genetic study in animals has shown the interaction between the genetic factor(s) and the dietary changes in producing diabetes. This is a laboratory duplication of what has been observed in nature among some ethnic groups with a specific pattern — the Yemenite and Kurdish new immigrants to Israel and the Bantus in South Africa — in whom the incidence of diabetes has risen with the change in their environment, and the change in their diet, consisting mainly of an increase in sucrose consumption. We have shown that there is an individual variation in the degree of carbohydrate metabolism impairment resulting from feeding on a high sucrose diet (6).

The necessity for the interaction between the genetic and dietetic factors in the development of diabetes, as demonstrated in the above experiment, explains why only a certain percentage of people develop diabetes on consuming large amounts of sucrose. Furthermore, the necessity for a long feeding period for the development of diabetes may explain in part why the incidence of diabetes rises with age.

Table IV Effect of intragastric loads of sucrose and starch, 0.5 g/100 g body wt., on the glucose blood level of normal rats fed stock diet

Load	No of animals	Fasting blood glucose, mg/100 ml	Excess blood glucose, mg/100 ml			
			30'	60'	90'	120'
Corn starch	10	87.2 ± 1.1	28 ± 2.5	29 ± 2.9	24 ± 2.9	14 ± 2.3
Sucrose	9	66.1 ± 1.2	53 ± 1.9	76 ± 2.0	61 ± 1.9	62 ± 3.1

Values are mean ± SE.

ready in 1874 that diabetic patients were able to utilize fructose given orally better than other sugars (12).

Several studies have shown that the rate of fructose elimination from the blood is the same in the diabetic as in the normal man. Liver vein catheterization studies have shown the same rate of fructose uptake in the splanchnic area in healthy subjects as in diabetic patients (8). Minkowski, in 1893 observed that fructose in contrast to glucose, led to glycogen formation in the liver when given to pancreatectomized dogs (16). However the effects of fructose and glucose infusions on the glycogen formation in liver and muscle have not earlier been studied systematically by direct technique in diabetic man. We have therefore made a series of experiments, trying to elucidate these problems, and some preliminary results are presented here. Corresponding studies in normal man have earlier been performed in our laboratory (2, 17).

EXPERIMENTAL PROCEDURES

Insulin was withdrawn 1 m the insulin-dependent patients 36 hours before the experiment. After an overnight fast glucose or fructose solutions were given intravenously to the subjects at a rate of 1 g per kg body-weight per hour. A control group of normal subjects was treated in the same manner.

Percutaneous liver and muscle biopsies were performed before and after the infusion, and the glycogen content was determined in the tissue samples (10). Glucose, lactate, pyruvate, and ketone bodies in blood were determined at intervals in all cases. In addition, the changes in the fructose concentration and in the acid-base balance in blood were followed during the fructose infusions.

RESULTS

Fructose infusion caused an increase of the liver glycogen content amounting to 35.3 g per kg wet weight. The increase was of the same magnitude in the normal subjects as

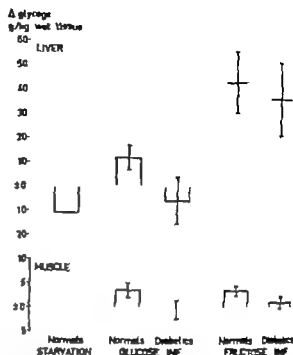


Fig. 2. Changes (mean \pm SD) in liver and muscle glycogen after i.v. fructose and glucose infusions (1 g/kg b.w./4 hrs) to normals and diabetics. The columns denote the changes from the values after an overnight fast. For comparison the mean decrease of the liver glycogen content after corresponding 4 hrs' period of starvation in normals is given.

in the diabetic patients. The muscle glycogen increase in normal subjects after fructose infusion was 3.3 g/kg as a mean (3). However in contrast to the findings in the normals, the muscle glycogen content in the diabetics showed no significant change after fructose (Fig. 1).

Glucose infusion caused a significant increase of the liver glycogen content in normal subjects of 11.3 g/kg liver. Contrarily to the increase of the liver glycogen in the normals, no change of the liver glycogen was observed in the diabetics in spite of very high blood glucose concentrations (500–1 000 mg per 100 ml). In the normal subjects the administration of glucose increased the muscle glycogen by 3.5 g/kg. The muscle glycogen level in the diabetic patients remained unchanged after glucose (Fig. 1).

The mean fructose level in blood during fructose infusion was in the normals 90 mg/100 ml and in the diabetics 120 mg/100 ml. The elevation of glucose in blood during fructose infusion was small in the normals but considerable in the diabetics. These observations are in agreement with previous studies (8).

Blood lactate increased rapidly and the increase was of the same magnitude in normals and in diabetics during fructose infusion. Concomitantly with the rise in blood lactate there was a fall in standard bicarbonate and pH in blood.

A rapid decrease of β -hydroxybutyrate and acetoacetate concentration in blood was observed both in the normals and the diabetics during fructose infusion.

Fructose excretion in urine was small and did not exceed 5 per cent of the infused quantity in diabetics or normal subjects. Fructose increased the urine glucose excretion in some of the diabetics but no glucosuria was found in the normals.

DISCUSSION

Several advantages for the clinical use of fructose have previously been presented (15-24). Some of these advantages are shortly summarized in the following. Fructose is metabolized without the presence of insulin. Fructose has a large distribution space and is rapidly metabolized, resulting in low blood concentration and a small urinary loss of the carbohydrate. Fructose decreases the plasma free fatty acids (16). The rapid increase of the respiratory quotient during fructose infusion, observed by many authors, can at least partly be due to a release of CO_2 from body fluids by the lactic acid formed during fructose metabolism. The protein sparing effect of fructose is a result of decreased gluconeogenesis from amino acids.

Complications due to fructose infusion have been reported. As mentioned before part of

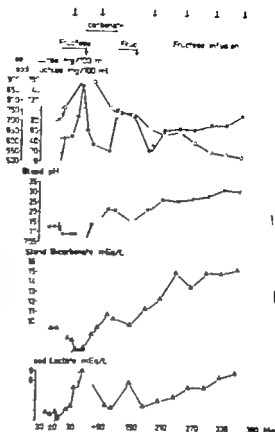


Fig 2 Fructose infusion to patient with severe keto-acidosis. The first fructose infusion was discontinued because of increasing acidosis. Insulin and bicarbonate were given iv. A continuous infusion of fructose could not be given till after repeated doses of insulin. In spite of insulin administration during the last fructose infusion blood lactate increased.

At each \downarrow 20 units of insulin was given iv

the fructose infused is transformed to lactic acid in the liver (2). Lactic acid gives rise to an acidosis, the degree of which is directly correlated to the infusion rate. In uncontrolled ketotic diabetics we have observed an aggravation of the condition of the patients, caused by the addition of a lactic acidosis to the keto-acidosis (Fig. 2) (3). The decrease of the bicarbonate concentration in blood through lactic acid formation from fructose was considerable. Insulin in combination with infusion of sodium bicarbonate in large

quantities may be necessary to improve this severe condition. The risk of provoking a lactic acidosis is a limitation for the use of fructose in diabetics with manifest or latent acidosis.

Liver and muscle glycogen are main stores of carbohydrate in the body. The formation of glycogen in the diabetic state after fructose or glucose infusion is still a controversial problem. There are three main steps for the transformation of hexose to glycogen in liver and muscle, which can be of interest for the discussion, i.e. the penetration of the sugar through the cell membrane, the phosphorylation, and the transformation from UDP-glucose to glycogen.

1. Glucose penetrates the liver cell independent of insulin while the penetration into the muscle cell is insulin-dependent (13). Fructose is not dependent on insulin for its penetration into liver or muscle (6).

2. The phosphorylation of glucose is brought about by hexokinase in liver and muscle and glucose is also phosphorylated

liver by a specific enzyme, glucokinase, which is insulin-dependent for its synthesis (3). The fructose phosphorylation in muscle by hexokinase is inhibited by glucose at normal concentrations according to data from animal experiments but evidence for a direct phosphorylation in human muscle of fructose under *in vivo* conditions have been presented (7). In the liver fructose is phosphorylated also by a specific ketohexokinase which is not dependent on insulin action (1) and not inhibited by glucose.

3. The transformation of UDP-glucose to glycogen is mediated by glycogen synthetase which appears in an active form (I) and an inactive form (D) (19). We have found the I activity and the total activity (I + D) to be reduced in muscle of untreated diabetic patients (20). No similar studies have as yet been made in human liver tissue. In normal experimental animals a conversion of the D to the I form has been demonstrated in liver

and in muscle after administration of glucose or of insulin (23). *In vitro* studies with fructose gave no D to I conversion in contrast to glucose (8).

According to our studies in untreated diabetic patients no increase of either synthetase I activity or glycogen synthesis occurs in muscle during glucose infusion. After insulin administration a conversion of the D to the I form is found concomitantly with a rise of glycogen (21). Similar results are reported by Villar Palasi et al. from studies *in vivo* of alloxan-diabetic rats (25). The liver of alloxan-diabetic rats has low activity of the I form, which does not increase during refeeding a carbohydrate rich diet that causes a substantial increase of the glycogen content (9). It has previously been suggested that glycogen synthetase is the rate-limiting enzyme for glycogen synthesis and that only the I form is active (7, 19). The D form is inhibited by physiological concentrations of ATP and inorganic phosphate (19).

The lack of glycogen formation in liver after glucose infusion in the diabetics can be explained by a low glucokinase and/or hexokinase activity or possibly by a low conversion of synthetase D to I. It is not probable that glycogen synthetase is the rate-limiting factor as the liver glycogen content in the diabetics was normal before the infusion. The block in glycogen synthesis from glucose is therefore more likely to be at the site of glucose phosphorylation.

In muscle already the entry of glucose into the cell is slowed by a defective penetration, which — like the enzymes involved in glycogen synthesis — is insulin-dependent (14).

The rapid formation of glycogen from fructose in diabetic liver indicates a normal activity of fructokinase and a sufficient synthetase activity for normal glycogen formation.

A pronounced decrease in ATP level due to fructose phosphorylation (11) could inhibit the synthetase D.

ACKNOWLEDGEMENTS

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METABOLIC EFFECTS OF DIETARY FRUCTOSE IN INSULIN DEPENDENT DIABETES OF ADULTS

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Abstract. The metabolic effect of an isocaloric replacement of 75 grams of dietary starch by fructose was studied in 10 insulin-dependent diabetic patients. Body weight, urinary excretion of glucose, diurnal variation of blood glucose, plasma free fatty acids (FFA) and plasma immunoreactive growth hormone (IRGH) and fasting levels of plasma cholesterol and triglyceride were determined during three successive dietary periods. During the first and third period starch was the main carbohydrate given while during the second period 75 grams of starch was replaced by fructose. Fructose feeding did not alter diurnal blood glucose or urinary output of glucose but tendency to an increase of plasma triglyceride and FFA levels was observed during the fructose period. Plasma levels of cholesterol and IRGH were unaffected by fructose.

The results suggest that in controlled insulin-dependent diabetes moderate amounts of fructose can be included in the diet without any adverse effects on the glucose balance. However it seems likely that triglyceride metabolism of diabetes is influenced by dietary fructose even in moderate doses.

Since the observations, one hundred years ago that in diabetic organism fructose is utilized better than glucose many investigations have been carried out to evaluate the

position of dietary fructose in the management of clinical diabetes (6, 11, 30, 14, for review see 7). There are, however, only few controlled studies where the metabolic effects of fructose and glucose have been compared (6, 18). The results showed that in mild and moderate diabetes isocaloric replacement of dietary glucose by a reasonable amount of fructose (up to 90 g) diminishes hyperglycemia and glucosuria. This beneficial effect of fructose is not seen in more severe forms of diabetes, however. The general conclusion of all these experiments has been that fructose as a therapeutic agent does not have any place in the treatment of clinical diabetes.

As pure fructose has now been made commercially available as a nutrient it was of interest to study whether insulin-dependent diabetic patients are able to use moderate amounts of this sugar without worsening of glucose or lipid metabolism.

MATERIAL AND METHODS

The dietary experiments were carried out in ten (five males and five females) insulin-dependent

diabetics selected from patients attending the Diabetes Outpatient Unit of the University Central Hospital of Helsinki. The median age was 25.5 years (range 18 to 70) and the relative body weight was within 90 to 110 per cent (mean 99). In all subjects the diabetes was of moderate degree the daily dose of insulin being from 22 to 44 units (mean 41) and the disease being relatively stable if not always well controlled with this dosage. The mean duration of the disease was 12.4 years (range 3 to 22). Seven patients had some signs of diabetic complications but none had uraemia (serum creatinine normal) or severe vascular disease.

All patients were taken to the metabolic ward for the whole study period. On admittance each was put on sugar free hospital diet the amount of which was adjusted to become isocaloric. Insulin was given as single injection of long acting preparation (Insulin Lente® Novo or Insulin Rapiard Novo) each morning and the dose was adjusted to give the best possible steady state condition in regard of body weight, blood glucose and urinary glucose output. It should be noted that a strict control of the diabetes was not attempted but just a stable condition with minimum day-to-day variations. One patient needed two daily injections of Insulin Lente®. The subjects were allowed a normal physical activity in hospital but no extra strenuous exercise.

After the dosage of insulin and body weight had been stabilized the dietary experiment was started. This consisted of three periods, ten days each. During the first and third periods the diet contained starch as the major carbohydrate (designated STARCH I and II) while during the second period 75 grams of starch was exchanged for the same amount of fructose (FRUCTOSE). In one patient the second starch period had to be omitted. The general outline of the study is shown in Fig. 1.

Starch I and II The diet during these periods was identical. It provided a total amount of 20 to 40 calories per kg per day and the approximate distribution of these was 40 per cent from carbohydrate, 40 per cent from fat (mainly saturated) and 20 per cent from protein. The carbohydrate was mainly starch (the total amount of oligosaccharides (lactose, sucrose, fructose) being about 25 grams daily). The diet was given as six meals between 8 a.m. and 8 p.m.

Fructose During this period 5 grams of fructose was added to the starch diet by simultaneously decreasing the calculated amount of starch to maintain the total carbohydrate intake

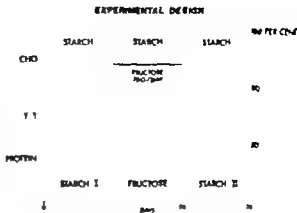


Fig. 1 Arrangement of dietary periods.

constant. The fructose covered thus an average amount of 40 per cent of carbohydrate calories and approximately 16 per cent of the total energy supply. Fructose was administered in five portions during the meals any single dose never exceeding 20 grams.

The following parameters were used as indicators of diabetic control.

Daily urinary excretion of glucose The quantitative excretion of glucose was determined by polariscope every day.

Diurnal level of blood glucose The diurnal variation of blood glucose was determined every third day by measuring the level of blood glucose every fourth hour during 24 hours beginning from 8 a.m. The patients were fasting from 9 p.m. to 8 a.m. and thus the eight o'clock morning values represent the fasting blood glucose.

Diurnal level of plasma free fatty acids (FFA) The diurnal variation of plasma FFA (24) was determined simultaneously with blood glucose in five patients. In the other five patients fasting level of plasma FFA (at 8 a.m. after overnight fasting) was determined every third day.

Diurnal level of plasma immunoreactive growth hormone (IRGH) The diurnal level of plasma IRGH (29) was determined simultaneously with the 4 h blood glucose curves in three patients.

Plasma cholesterol and triglyceride Fasting levels (after overnight fasting) of plasma cholesterol (24) and triglyceride (23) were determined every third day in eight patients.

Body weight Body weight was measured every day.

Urinary ketone bodies Keturia was assessed by Acetest reagents in each sample of urine containing glucose.

Table I. Urinary output of glucose, fasting blood glucose and body weight (mean \pm S.E.) in 10 insulin dependent patients during three successive dietary periods

	Starch I	Fructose	Starch II
Urinary glucose g/day	17.3 \pm 5.3	16.7 \pm 4.5	12.9 \pm 3.3
Fasting blood glucose mmol/L	7.9 \pm 0.6	8.6 \pm 0.6	7.7 \pm 0.7
Body weight kg	60.6 \pm 2.6	59.8 \pm 2.5	59.5 \pm 2.4

Diurnal level of serum fructose (3) was also measured simultaneously with blood glucose in three patients.

For fasting blood glucose, FFA, cholesterol and triglyceride the mean values were calculated for each patient and dietary period. The results are presented as mean values of all patient means \pm s.e. for each of the three periods.

RESULTS

All patients tolerated the fructose well without developing any side effects. Thus, none suffered from diarrhoea, which often occurs with higher daily intakes of monosaccharides obviously as an osmotic effect. Most patients did not like the sweet taste of fructose. In spite of attempts to keep the diet at isocaloric level some patients reduced weight during the three experimental periods and the mean fall of 1.1 kg per 30 days occurred as is apparent from Table I. The insulin dosage remained constant through all three dietary periods in all subjects except one, in which reduction by 4 units was necessary during fructose period because of morning hypoglycemia.

Blood fructose. The levels of fructose in peripheral venous blood were too low to be accurately determined by the method used. The order of magnitude was 3 mg/100 ml

BLOOD AND URINARY GLUCOSE

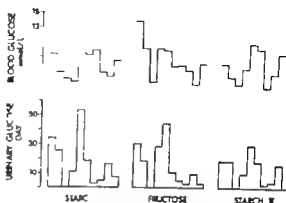


Fig. 2. Mean excretion of urinary glucose and mean levels of fasting blood glucose in ten insulin dependent patients during three successive dietary periods.

or less and in none of the single determinations carried out from samples taken around the day did fructose concentration exceed a value of 10 mg/100 ml.

Glucose balance. The mean values of fasting blood glucose and of daily urinary excretion of glucose during the three dietary periods are presented in Table I. It is seen that all values were closely similar and there was no statistical difference in these parameters between the fructose and starch periods. Fructose feeding did not influence the fasting blood glucose or urinary glucose irrespective of whether the diabetic control was excellent or poor in the beginning of the experiment (Fig. 2). Thus, in patients with less strict control before the experiment the glucose status was not essentially worsened during the fructose period. Ketonuria was detected only in a few instances and there was no difference between the periods in this respect.

The diurnal blood glucose curves are presented in Fig. 3. All three curves are virtually identical indicating that fructose did not influence the diurnal variations or mean level of blood glucose.

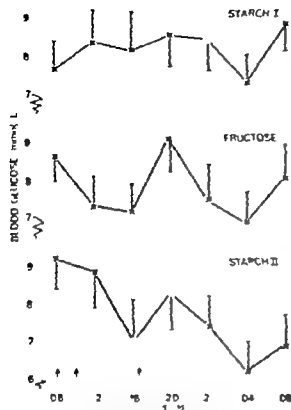


Fig. 3 Diurnal variation of blood glucose (mean \pm S.E.) in ten insulin dependent diabetic patients during three successive dietary periods. The arrows indicate meals.

Lipid metabolism. All patients had plasma cholesterol and triglyceride levels within normal range at the start of the dietary experiments. The mean values of these lipids as well as of fasting plasma FFA during the three periods are presented in Table II. Cholesterol level did not show any definite trend during fructose period as compared to starch but plasma triglyceride concentration tended to increase on fructose even though the difference between the means of fructose and starch periods is not significant. The fasting plasma FFA during fructose feeding was significantly ($p < 0.05$) higher than during any of the two starch periods. On the other hand, the diurnal variations of plasma FFA determined in five patients were not essentially different during the three

Table II. Fasting levels of plasma cholesterol and triglyceride (mean \pm S.E.) in eight and plasma FFA (mean \pm S.E.) in ten insulin dependent diabetic patients during three successive dietary periods

	Starch I	Fructose	Starch II
Cholesterol (mmol/L)	4.99 ± 0.1	5.10 ± 0.11	5.26 ± 0.13
Triglyceride (mmol/L)	1.00 ± 0.06	1.32 ± 0.08	1.05 ± 0.14
FFA (mmol/L)	0.61 ± 0.03	0.71 ± 0.04	0.50 ± 0.03

$p < 0.05$

dietary periods (Fig. 4). During fructose there was a tendency to lower plasma FFA after noon and night values as compared to starch but these differences were not significant.

Plasma growth hormone (IRGH). The mean diurnal level of plasma IRGH determined in three patients was very similar during the three dietary periods (Table III). The content of plasma IRGH did not fluctuate more during the fructose than during the two starch periods.

DISCUSSION

The metabolic pathway of fructose is in many respects dissimilar to that of glucose. The main bulk of ingested fructose is taken up by the liver and converted to trioses (15). A part of the ingested fructose is either converted to glucose in the intestinal wall, or taken up by adipose and muscle tissues (4, 5, 2, 17). The quantitative distribution of fructose assimilation between the various tissues is, however not exactly known. Rapid metabolism of fructose to triose units occurs also in a diabetic liver (16, 17). The subsequent fate of trioses generated from fructose in diabetic organism apparently depends on the degree of insulin deficiency. If gluconeogenesis dominates as in severe diabetes increased production of glucose is the result while in controlled diabetes fructose is con-

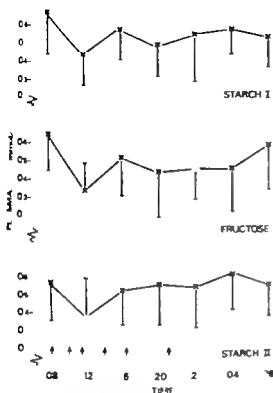


Fig. 4. Diurnal variation of plasma FFA (mean \pm S.D.) in five insulin-dependent diabetic patients during three successive dietary periods. The arrows indicate meals.

verted to pyruvate and other products of Krebs cycle. Since diabetic organism is capable for utilizing pyruvate fructose can really provide fuel to energy metabolism without increase of blood glucose (27).

In the present investigation the diabetes was in all patients at least moderately controlled. the mean excretion of urinary glucose varied from 0 to 53.7 g/day and the mean levels of fasting blood glucose varied from 5.6 to 10.9 mmol/L and none of the patients was ketotic. Therefore it was not unexpected that under these conditions replacement of 75 grams of dietary starch by an equal amount of fructose and given in small portions did not influence blood or urinary glucose. The results obtained are in concordance with the earlier observations that small amounts of

Table III The mean 24 hour-content of plasma IRGH (mean \pm S.E.) in three insulin dependent diabetic patients during three successive dietary periods

Diet period	Plasma IRGH ng/ml
Starch I	3.28 \pm 0.58
Starch II	3.86 \pm 0.55
Fructose	3.68 \pm 0.62

fructose do not impair the diabetic control (6 14 18 30)

The effect of dietary fructose on plasma lipids is important when the use of fructose as a dietary component of diabetic patients is considered. In experimental animals fructose exerts a more pronounced hyperglyceridemic effect than glucose (1, 20 31). Moreover oral fructose enhances exogenous hyperglyceridemia in rat and man (21 22). The hyperlipidemic effects of isocaloric exchange of various dietary carbohydrates (starch, sucrose, malt, glucose fructose) are on the other hand somewhat contradictory as has been reviewed recently by Nikkilä (9—13 19 25). No previous data are available on the effect of fructose feeding on plasma lipids in diabetic man, however. It is obvious that in insulin deficiency whether corrected by exogenous insulin or not, modifies the interaction of carbohydrate feeding and lipid metabolism since the insulinogenic stimulus exerted by the ingested carbohydrates is lacking. In the present investigation the tendency to develop hyperglyceridemia is noteworthy since the duration of the dietary periods may have been too short for an effective induction of hyperglyceridemia by the relatively small doses of fructose used. Furthermore, it is possible that the slightly negative caloric balance present in many cases masked to some extent the fructose effect.

A somewhat unexpected finding was the increase of fasting plasma FFA during the fructose period. No adequate explanation can

be offered for this phenomenon so far. In nondiabetics this could be attributed to the lesser stimulation of insulin secretion by fructose and concomitant increase of lipid mobilization from adipose tissue but the present diabetic patients were all receiving a constant dose of exogenous insulin during all three periods. The possibility of morning hypoglycemia and associated catecholamine response is excluded by the close similarity of the diurnal blood glucose curves during the fructose and starch periods. Since the elevated basal FFA level during fructose-rich diet may form one additional factor for the development of hypertriglyceridemia it should be of interest to confirm the present finding by a more extensive study including also nondiabetic subjects.

There were two reasons to include the plasma IRGH determinations into the present study. Firstly it has been shown that in diabetic patients plasma IRGH level is higher during poor than during good control (8) and, secondly because intravenous fructose has recently been postulated to stimulate growth hormone release (26). In the present experiments however that small oral doses of fructose did not influence the plasma level of IRGH.

The data accumulated in this investigation thus revealed that in short term experimental conditions isocaloric replacement of dietary starch by 73 grams of fructose does not influence the diabetic control as judged by the levels of blood and urinary glucose. The results suggest that the most critical point in the chronic use of fructose in diet can be the effect of fructose on lipid metabolism.

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DOES DIETARY FRUCTOSE AFFECT THE CONTROL OF DIABETES IN CHILDREN?

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Abstract. The purpose of the study was to find out whether fructose can be used as a dietetic sweetener for diabetic children.

Acute effects of ingested fructose were studied in 28 hospitalized diabetic children by giving fructose, 1.8 g/kg, at breakfast, and by comparing 3 hours postcibal blood glucose to that of control days, when a corresponding amount of calories was given as a regular breakfast. Postcibal hyperglycemia from 30 to 120 min was significantly less on fructose days as compared with control days. The differences between blood glucose on control and fructose days were significantly greater from 30 to 120 min than the chance variations on two control days.

The effects of more prolonged use of dietary fructose on diabetes were studied in 18 patients at home for four weeks by alternating between one week periods on diets which included 15 g/kg/day of fructose and control weeks of regular isocaloric sugar free diets. Glucosuria, recorded twice daily by Clinitest and once a week by measuring 24-hour glucose excretion, was similar during control and fructose weeks. Ketonuria was infrequent during either diet. Fructose did not elevate serum triglycerides.

Because dietary fructose, given in substantially larger amounts did not impair the control of diabetes, it is concluded that fructose can be used by diabetic children at rate 1.8 g/kg/day without negative effect so long as the patient

otherwise adheres to a quantitative diet, includes fructose on an isocaloric basis, and only parent/mother administers the compound.

The fate of infused or ingested fructose in the diabetic organism has long aroused interest. Particularly the tolerance to dietary fructose has been the subject of numerous studies, ever since the early report by Kälz (10) of a decrease in diabetics' glucosuria after incorporation of fructose in the diet.

However the fructose tolerance of diabetic children has not been sufficiently investigated so far. To explore whether dietary fructose affects the control of diabetes in children, controlled experiments were carried out. In one part of the study patients were given fructose as an acute load at breakfast, and, in another part, fructose was administered throughout the day during two one-week periods. The effect of fructose on the diabetic state was judged in the former group from postcibal blood glucose changes, and in the latter group from glucosuria and ketonuria.

Table 1. Composition of breakfast given to 26 diabetic children on control and fructose days

Control day(s). Regular breakfast for diabetics		Fructose day Breakfast containing fructose 1.0 g/kg/body wt	
Porridge	100–150 g	Chocolate + Cream	10 g 10–15 g
Milk	100–150 g	or Lemon juice	20–30 g
Bread	10–15 g	Bread	10–15 g
Butter	2.5–7 g	Butter	2.5–7 g
Meat (veal)	20–40 g	Meat (veal)	20–45 g
Orange juice	0–100 g	Egg (boiled)	one
CHO	45 g	CHO	45 g
Fat	35	Fat	35–37
Protein	40 g	Protein	20–18
Kcal, mean \pm SEM	405 \pm 16	Kcal, mean \pm SEM	416 \pm 11
20 % of total daily calories			

PATIENTS AND METHODS

The data were obtained from 51 diabetic children. The patients (23 boys, 28 girls) ranged in age from 2 to 16 years, the mean age being 10 years. They were treated with intermediate-acting insulin, given once or twice daily. Fructose was given either as an extra load, 1.0 g/kg weight at breakfast (series A) or in an amount of 1.5 g/kg body weight/day divided into several portions throughout the day during two one-week periods (series B).

Series A. Twenty-six diabetic children, hospitalized either because of newly detected diabetes or due to poor control of previously diagnosed disease were given fructose 1.0 g/kg body weight at breakfast. The experiments were done in the cases with recent onset (14 children) at the end of the initial stabilisation period shortly before discharge and in the cases which had been admitted due to poor control (12 children), after some improvement had been achieved. The composition of the isocaloric breakfast on control and fructose days is given in Table 1. Fructose was included in chocolate or lemon juice. On the fructose days the majority of calories provided by carbohydrates in the breakfast came from fructose.

The diet during the rest of the day was an ordinary diet for diabetic children. The fructose day was either the day following the control days or the day between them. The dose and time of administration (30 min before breakfast) of insulin remained unchanged for each patient

during the control and fructose days. Capillary blood specimens for blood glucose determination were taken immediately before breakfast and 30, 60, 90, 120 and 180 min after it. The mean value of the two control days for each sampling was used in the comparison with the fructose day value. In seven cases we have data from one control and one fructose day. Forenoon snack was combined with breakfast, and therefore the caloric content of the latter rose to 20 per cent of that of the day (Table 1). The last blood specimen was taken before the patients had their luncheon. Blood glucose was measured by a commercial glucose oxidase method (Kabi Reagents, Stockholm). Blood fructose, lactate and pH on the fructose day were determined in five and serum triglycerides (at 60 and 120 min) in six patients. Fructose was analysed by an anthrone procedure (4), and the interference of blood glucose was eliminated by correction. Lactate was determined by a commercial lactate dehydrogenase method (Biochemicals Boehringer Mannheim), pH by the Astrup micro technique and triglycerides fluorometrically (16,9).

Series B. The children were studied at home during a period of 28 consecutive days. All patients had had diabetes for more than six months and they had passed the partial remission. The diet during the first and third weeks (control) was regular diet for diabetic children sugarfree 1000 kcal + 100 kcal/year daily and proportion of calories derived from carbohydrate and protein were 45, 35 and 20 per cent, respectively. The diet during the second and fourth weeks (fructose) was isocaloric with the

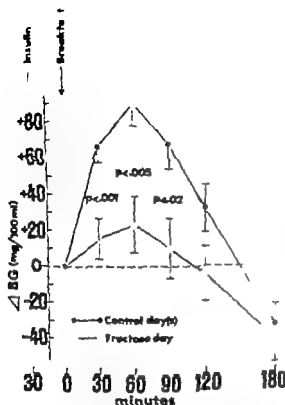


Fig. 1 Changes in blood glucose (mg/100 ml) mean \pm SEM after a regular breakfast for diabetic children, and an isocaloric fructose-containing breakfast in 26 diabetic children. Fructose dose 1.0 g/kg body wt.

diet of the control weeks, but a part of the carbohydrates from starch was substituted for by fructose at a rate of 1.5 g/kg/day and corresponding to about 20 per cent of the daily carbohydrates. Fructose was administered in beverage (10 g/100 ml) which the children drank at meals and snacks. The actual daily intake of carbohydrates was similar from 203 \pm 8 (mean \pm SD) to 215 \pm 9 g during both types of weeks. The patients' mothers received diet lists for each day of the observation period, sheets for recording the actual food intake, and were instructed in detail on dietary matters before the study. The insulin dose of each patient remained constant during the study and they were advised to exercise regularly and to the same extent during control and fructose weeks. The patients and/or their mothers were checked at the start of the study as regards their ability to do Clinitest and Ketostix tests properly. One of the investigators made home calls during the ex-

perimental period, re-checked the urine testing and discussed the dietary instructions. Clinitest®

Ketostix® tests were made every morning the patient's second morning urine specimen, the evening before bed time snack. For each patient the mean value of the daily Clinitest and Ketostix for each one week period was compared morning and evening specimens taken. At the end of each week 24-hour urine

specimens were collected, brought to the laboratory, studied for glucosuria by polarimetry and for ketonuria by Ketostix®. Several patients had to be omitted from the series because of concurrent acute infections, and a few had difficulties in adhering to diet prescriptions or to inaccurate urine testing. Data on 26 patients are reported. Fasting venous blood glucose were drawn from some of these children at the end of each week for the determination of serum triglycerides.

The significance of differences between mean values was calculated by Student's *t* test, and between individual observations by paired comparison. The differences in blood glucose values in series A between control and fructose days from corresponding specimens were compared to the chance variations of blood glucose of the same hour on two control days by the matched pair Wilcoxon test (1).

RESULTS

Series A.

Blood glucose (BG). Mean changes in BG after breakfast on control and fructose days are shown in Fig 1.

A paired comparison showed smaller postprandial increases in BG values on fructose day compared to control days from 30 to 120 min. ($p < 0.01$, < 0.001 , < 0.005 and < 0.05 at 30, 60, 90 and 120 min. respectively). It is well known that in diabetic children BG at a certain time of the day may vary considerably from day to day. In this study however the differences between BG values on control and fructose days were significantly greater from 30 to 120 min. than the variations on two control days.

When the BG results of the subjects with recent onset of diabetes and of those admitted due to poor control were studied

Table II. Changes in blood glucose (mean \pm SEM) after a regular breakfast for diabetics, and an isocaloric fructose-containing breakfast in 14 diabetic children, admitted due to onset of the disease

Fructose dose 1.0 g/kg body wt

	Δ 30 min	Δ 60 min	Δ 90 min mg/100 ml	Δ 120 min	Δ 180 min
Control day(s)	59 \pm 11	80 \pm 19	50 \pm 21	8 \pm 20	-56 \pm 20
Fructose day	19 \pm 17	28 \pm 23	12 \pm 23	-7 \pm 23	-43 \pm 18

Table III. Changes in blood glucose (mean \pm SEM) after a regular breakfast for diabetics, and an isocaloric fructose-containing breakfast in 12 diabetic children, admitted because of poor control of the disease

Fructose dose 1.0 g/kg body wt

	Δ 30 min	Δ 60 min	Δ 90 min mg/100 ml	Δ 120 min	Δ 180 min
Control day(s)	73 \pm 14	102 \pm 18	67 \pm 14	60 \pm 14	-7 \pm 10
Fructose day	11 \pm 18	18 \pm 23	8 \pm 23	-2 \pm 22 ^b	-37 \pm 19

^a $p < 0.01$ vs control day(s)

^b $p < 0.05$ — —

separately significantly lower BG increases after breakfast on fructose days compared to control days were observed only in the latter group of patients (Tables II and III).

The mean 0-values did not differ from each other on control and fructose days in either group of diabetics in cases with recent onset BG at 0 min. was 169 ± 13 (SE) on control and 163 ± 20 mg/100 ml on fructose days, and in cases with previously diagnosed diabetes 185 ± 20 on control and 207 ± 3 mg/100 ml on fructose days.

Blood fructose The mean blood fructose levels before and after a fructose-containing breakfast in five diabetics are given in Table IV

Blood lactate and pH Mean blood lactate values of five patients are seen in Table IV. Hyperlactatemia paralleled the rise in blood fructose. Blood pH remained unchanged after fructose ingestion in the same subjects despite the rise in blood lactate.

Serum triglycerides Levels of serum

triglycerides in the six patients studied were unaffected by fructose load. The mean value was 1.15 ± 0.31 (SE) at 0 min., 1.10 ± 0.27 at 60 min., and 1.16 ± 0.26 mmol/l at 120 min.

Series B

Glucosuria. The average concentration of glucose in urine measured at home by Clin-Itect[®] from morning and evening specimens is shown in Fig. 2.

Relative glucosuria was virtually identical during control and fructose weeks. Quantitative glucose excretion at the end of each week is given in Table V.

Differences in glucosuria were found neither between group mean values during control and fructose weeks, nor in an individual paired comparison. Clin-Itect[®] recordings done in the laboratory on the 24-hr urine specimens were generally in good agreement with the results obtained with polarimetry.

Table IV Blood fructose and lactate (mean \pm SE) of 7 patients receiving a fructose-containing breakfast in five diabetic children

Fructose dose 10 g/kg body wt

	0	30 min	60 min	90 min	120 min
Fructose, mg/100 ml	3.2 \pm 0.2	13.4 \pm 1.8	8.2	5.0 \pm 0.0	4.0 \pm 0.4
Lactate, mg/100 ml	0.3 \pm 1.0	20.0 \pm 0.3		14.0 \pm	1.3 \pm 1.4

p < 0.001 vs 0

p < 0.005

p < 0.02

p < 0.05

Table V Glucosuria at the end of each week receiving dietary fructose 1.5 g/kg/day every 4 hr

Diets were isocaloric during control and fructose

	I week Control n = 15	II week Fructose n = 15	III week Fructose n = 15	IV week Fructose n = 15
		Glucosuria, g, % per 24 hr \pm SEM		
8 a.m.—8 p.m.	24.2 \pm 0.5	18.3 \pm 5.0	3.2 \pm 1	5.0
8 p.m.—8 a.m.	15.5 \pm 3.9	16.9 \pm 6	4.1 \pm 8	—
		(14)		
Per 24 hours	39.7 \pm 8.8	35.7 \pm 9.3	34.4 \pm 16	4

Ketonaemia. Ketonaemia, as measured by Ketostix® was infrequent during both types of diets. A few patients only had one positive recording out of 16 specimens analysed per week. A 2 year old boy had 5/16 positive recordings during the first control week, and thereafter 1/16 positive recordings during the subsequent fructose and control weeks.

The control of diabetes in the patients of series B before the trial with dietary fructose was on an average only fair as seen from the mean Clinitest® recordings of 1.2 and 1.5 % during the first week (Fig. 2) and the average glucosuria of approximately 40 g/24 hr at the end of the first week (Table V).

Serum triglycerides. Fasting serum triglycerides, (mean \pm SE, mmol/l), at the end of each week were as follows: first week (control) 1.02 \pm 0.10 (n = 7) second week (fructose) 0.91 \pm 0.08 (n = 7) third week (control) 0.88 \pm 0.05 (n = 9) and fourth week

(fructose) 0.82 \pm 0.07 (n = 6). Thus, no increase occurred.

Side effects

One diabetic in series B had diarrhoea after drinking the fructose-containing beverage. This ceased when the patient was given fructose in desserts and bakery. Several children in both series stated that the fructose drinks tasted too sweet.

DISCUSSION

Fructose is absorbed from the small intestine in linear relation to the concentration of the sugar in the intestine (3). Although human mucosal cells contain the enzymes necessary for the conversion of fructose to glucose, such a transformation during the absorption through the intestinal wall in man is considered unlikely (5) or occurs only to a slight degree (3, 14).

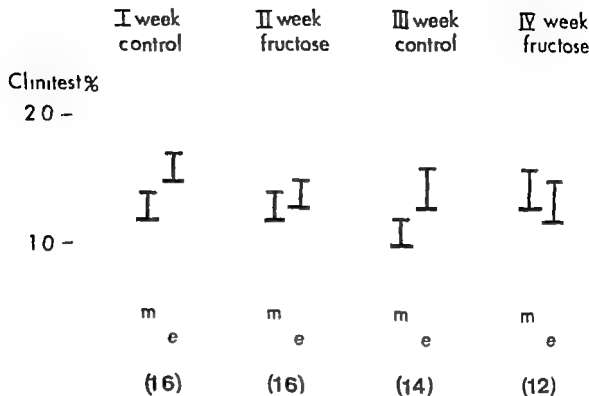


Fig. Relative glucosuria (%), mean \pm SEM as measured by Clinitest® at home from morning (m) and evening (e) specimens of diabetic children receiving dietary fructose 1.5 g/kg/day every second week. Diets were isocaloric during control and fructose weeks. The number of subjects is indicated in brackets.

In the liver fructose is rapidly metabolized to triose level by insulin-independent enzymes (20). The liver of diabetics can take up fructose without difficulty even in severe insulin deficiency (14). From the trioses the path goes either to glycolysis or to gluconeogenesis: glyceraldehyde is metabolized to lactic acid, and dihydroxyacetone phosphate can be metabolized via the Embden-Meyerhof pathway to glucose and glycogen. Tracer studies indicate that a significant proportion of fructose entering the liver is converted to glucose in normal man (1).

Fructose-1,6-diphosphatase is one of the key-enzymes in gluconeogenesis (17). Its

activity is increased in diabetes during insulin deficiency contributing to augmented gluconeogenesis from different precursors. The unimpaired hepatic uptake of fructose may therefore result in an increased hepatic release of glucose. Some clinical reports on the intravenous or oral use of fructose in uncontrolled diabetes confirm the accelerated formation of glucose from fructose (8, 13, 15). Relative insulin deficiency occurs in childhood diabetes at times despite treatment, and therefore fructose administration to these patients might theoretically lead to impaired control of the disease.

Fructose has been widely used in some countries as a substantial constituent of the diet or as a sweetening agent by adult diabetics without deterioration of the control of the disease as reviewed by Mehnert (17). The daily dose has usually been 60–90 g or less, and the postulated advantages are slow absorption, utilization without insulin and antiketogenic effect. The slighter the diabetic state the greater are the benefits of fructose administration (12).

Only a few reports seem to be available on the effect of dietary fructose on the control of diabetes in children. Hartmann et al. (7) gave about half of the daily carbohydrates as fructose to five children for several weeks to see if an insulin-sparing effect would appear as compared to isocaloric glucose or sucrose feeding. The patients were in good or fair control during the study. The authors found no insulin-sparing effect of dietary fructose. Rosenkranz and Wagner (18) gave an acute fructose load of 1.5 g/kg to nine children after the morning insulin and one hour after breakfast. The BG values ranged between 120 and 250 mg/100 ml before fructose administration. A decrease in BG was seen in eight patients within 90 min.

The present study was made to find out whether or not fructose could be used as a sweetener of food for diabetic children. For sweetening of food considerably smaller doses of fructose than we employed in either part of the investigation are sufficient. The fairly large doses of fructose were chosen to make it easier to reveal effects, if any on the parameters used as criteria for the control of diabetes.

The significant differences in the acute experiment between postcibal BG changes on fructose and control days (Fig. 1) are difficult to explain. Fructose is absorbed slower than glucose at low concentration, whereas at a high concentration fructose and glucose are absorbed to a similar degree (8). Because the fructose concentration of the fructose day breakfast was high, fructose was presumably absorbed more rapidly than was glucose which was derived from slowly digested starch on control days. We cannot therefore attribute the milder hyperglycemia on fructose days to slow absorption. The diabetes of our subjects in series A was on the average under fair control, as seen from the mean BG levels at 0 min. (half an hour after the administration of insulin) on the experimental days. The difference between

fructose and control days in series A. BG never was greater than the patients had before. In series B the postcibal BG was higher than on control days. This was due to the fact that the patients in series B were not under fair control.

part of the study was made to find out whether or not fructose could be used as a sweetener of food for diabetic children. For sweetening of food considerably smaller doses of fructose than we employed in either part of the investigation are sufficient. The fairly large doses of fructose were chosen to make it easier to reveal effects, if any on the parameters used as criteria for the control of diabetes. The significant differences in the acute experiment between postcibal BG changes on fructose and control days (Fig. 1) are difficult to explain. Fructose is absorbed slower than glucose at low concentration, whereas at a high concentration fructose and glucose are absorbed to a similar degree (8). Because the fructose concentration of the fructose day breakfast was high, fructose was presumably absorbed more rapidly than was glucose which was derived from slowly digested starch on control days. We cannot therefore attribute the milder hyperglycemia on fructose days to slow absorption. The diabetes of our subjects in series A was on the average under fair control, as seen from the mean BG levels at 0 min. (half an hour after the administration of insulin) on the experimental days. The difference between

Dietary fructose elevates serum triglycerides in nondiabetic men and post menopausal women (11). Fructose ingestion did not raise the serum triglycerides of our diabetic subjects, and particularly not in the cases of two boys who had reached puberty.

Our results do not give an answer to the question of the tolerance of dietary fructose, if consumed for months or years. In older literature the opinion prevailed that a long lasting use of fructose would lead to impaired utilization in diabetics in the course of time. Contrary to this assumption, Schar-

tow (18) found no change in the fructose tolerance of 89 adult diabetics, who took fructose 3 X 10 g daily for 18 months.

Because the diabetic state was not impaired in either part of our investigation, we conclude that in diabetic children, whose disease is in good or fair control, fructose can be used, at least for short periods, as one alternative for sweetening of food (desserts, bakery etc.) provided that

- The child adheres to a quantitative diet.
- Fructose is used on isocaloric basis.
- Only the child's mother administers the compound.

If fructose is used, the mother should be carefully instructed about the necessary calculations for the diet. For sweetening purposes, less than 1 g for instance 0.5 g/kg body weight/day is sufficient.

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FRUCTOSE AND LIPID METABOLISM

THE EFFECT OF FRUCTOSE ON HEPATIC SYNTHESIS OF FATTY ACIDS

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Abstract The synthesis of fatty acids from fructose and glucose has been compared in rat and human liver. In both species fructose is a better precursor of fatty acids than glucose, primarily as a result of the greater rate of glycolysis of fructose as compared to glucose. The high rate of fructose utilization and relatively low rates of glucose utilization by liver is reflected by the activity of fructokinase which is considerably greater than the total hexokinase and glucokinase activities. Although it is not possible to exclude that the high rate of fructolysis produces metabolic intermediates which alters the functional state of the fatty acid synthesizing enzymes, the experimental evidence indicates that hepatic fatty acid synthesis in carbohydrate-fed animals is limited by the supply of substrate. Because the hepatic metabolism of fructose can supply acetyl-CoA to the fatty acid synthesizing enzymes at greater rates than can glucose, hepatic fatty acid synthesis is enhanced in fructose- or sucrose-fed animals. Since estimates of maximum rates of fatty acid synthesis in human liver based on *in vitro* measurement of the activity of acetyl-CoA carboxylase suggest that it is more than 10 times greater than the turnover of triglycerides in very low density lipoproteins, the data are compatible with the conclusion that enhanced rates of hepatic fatty

acid synthesis contribute significantly to fructose- or sucrose-induced hypertriglyceridemia in normal men.

The observation that substitution of isocaloric amounts of sucrose for fructose in the diet increases the concentration of plasma triglycerides in man and experimental animals (2, 3, 7, 10—13) has stimulated widespread interest in the possible relationships between the source and amount of sugar in the diet and the genesis of premature vascular disease (3, 22). Our own interest in this problem has focused on the possibility that the principal mechanism for the selective effects of different sugars on the concentration of plasma triglycerides is via altering the rate of hepatic fatty acid synthesis (7, 19, 23—29). Since sucrose is hydrolyzed to glucose and fructose during intestinal absorption, and since fructose when fed to rats has an identical effect on plasma triglyceride con-

Table I. Hepatic activities of acetyl-CoA carboxylase and fatty acid synthesis in fructose and chow fed rats. Activities are expressed as μ moles substrate metabolized per min per mg protein. Data from Zakim and Ho (27)

Diet	Acetyl-CoA Carboxylase	Fatty Acid Synthesis
Chow	2.20	2.96
Fructose	4.87	10.86

fatty acid synthetase to levels greater than those seen in animals fed a standard chow diet. Thus, one of the ways in which fructose feeding can enhance the hepatic synthesis of fatty acids is by increasing the amounts of fatty acid synthesizing enzymes. It is known, however, that glucose feeding may have a qualitatively similar effect on fatty acid synthesizing enzymes. For this reason a direct comparison of the effects of feeding large amounts of glucose or fructose on the activities of fatty acid synthesizing enzymes was carried out. For these experiments rats were fed diets containing 80 per cent of calories as fructose or glucose for two days. At the end of this period animals were killed and the activities of three fatty acid synthesizing enzymes measured. As seen in Table II fructose and glucose feeding had quantitatively identical actions on the activities of acetyl-CoA carboxylase citrate cleavage enzyme, and NADP malate dehydrogenase increasing each by 2 to 3-fold. Although the activity of fatty acid synthetase was not measured in this study the activity of acetyl-CoA carboxylase is rate limiting for the synthesis of fatty acids in the presence of saturating amounts of substrates (8, 14, 15). These data indicate, therefore, that although fructose-induced increases in the activities of fatty acid synthesizing enzymes in rat liver can contribute to fructose-induced hypertriglyceridemia, they cannot account for the differential effects of this sugar as compared

Table II. Comparison of the effect of fructose and glucose feeding on the activities of hepatic fatty acid synthesizing enzymes. Activities are expressed as μ moles substrate metabolized per min per mg protein. Data from Zakim et al. (28)

Diet	NADP Malate Dehydrogenase	Citrate Cleavage	AcCoA Carboxylase
Purina Chow	8.73	3.55	8.19
Glucose	23.3	10.40	8.78
Fructose	23.1	10.73	9.60

to glucose. It should be pointed out, however, that since large amounts of glucose increase the maximum capacity for hepatic fatty acid synthesis, variations in the glucose content of the diet may also be associated with changes in the concentration of plasma triglycerides in normal animals.

The conversion of 14 C fructose and 14 C-glucose to fatty acids in liver slices

Intravenous administration of fructose as compared to glucose is associated with a large increase in the concentration of plasma pyruvate (12). Studies with liver slices also document that fructose is metabolized more rapidly than glucose, and that these differences cannot be accounted for by substrate induction of specific fructose metabolizing enzymes (16). It appeared important, therefore to investigate the conversion of fructose and glucose to hepatic fatty acids using experimental conditions which did not allow for alteration of the amount of enzymes along the pathway from fructose or glucose to fatty acids. For these studies the conversion of 5.0 mM 14 C fructose or 5.0 mM 14 C-glucose to fatty acids was measured in liver slices incubated in Krebs-Henseleit solution (28). The production of 14 CO₂ was also monitored. In animals fed a normal diet of Purina rat chow liver slices converted ap-

Table III. Comparison of the conversion of ^{14}C fructose and ^{14}C -glucose to fatty acids and CO_2 in liver slices from rats fed a chow diet. Values given for products formed are the mean \pm SE of $\mu\text{moles } ^{14}\text{C}$ fructose or ^{14}C -glucose recovered per 100 mg tissue per 3 hour incubation. Tissue from 8 different rats was used for each experiment. Data from Zakim et al. (28)

Product	Labelled Substrate	
	^{14}C Fructose	^{14}C -Glucose
Fatty Acids	16.8 ± 8.1	3.7 ± 1.5
CO_2	902.4 ± 93.4	170.3 ± 20.9

proximately four times more ^{14}C -fructose than ^{14}C -glucose to fatty acids (Table III) indicating that fructose was a superior precursor of fatty acids than was glucose, in experiments in which there was no contribution to fatty acid synthesis from inductive effects on the amounts of fatty acid synthesizing enzymes. A similar result was obtained in studies with human liver slices. The data in Table IV data from a typical experiment that in the human liver fructose is a better precursor of fatty acids than is glucose. Also, more ^{14}C fructose than ^{14}C -glucose was converted to CO_2 , glycogen, and glyceride-glycerol in these slices.

The above experiments indicated that differences in the dynamic regulation of fructose and glucose metabolism were responsible for the varying effect of these sugars on hepatic fatty acid synthesis. However in view of the data in Tables I and II which suggest that diet can influence the rate of hepatic fatty acid synthesis by inductive effects on the amounts of fatty acid synthesizing enzymes, the influence of prior feeding of 80 per cent of calories as fructose or glucose on the subsequent conversion of ^{14}C -sugars to fatty acids was also studied in rat liver slices. For obvious reasons, it was not possible to do similar experiments with human liver. As expected from the data in Tables I and II feeding the high glucose

Table IV. Comparison of the conversion of 5.0 mM ^{14}C fructose and ^{14}C -glucose to fatty acids, glyceride-glycerol, CO_2 and glycogen in human liver slices. Values given for products formed are $\mu\text{moles } ^{14}\text{C}$ fructose or ^{14}C -glucose recovered in each product per 100 mg tissue per 30 min incubation and are the means of duplicate incubations. All data in this Table are from the same liver and are taken from Zakim et al. (26)

Product	Labelled Substrate	
	Fructose	Glucose
Fatty Acids	43.0	3.5
Glyceride-Glycerol	50.3	15.8
CO_2	337.0	70.4
Glycogen	69.3	5.1

or fructose diets increased the conversion of both ^{14}C fructose or ^{14}C -glucose to fatty acids. (Tables III and V) However irrespective of the antecedent diet, more ^{14}C -fructose than ^{14}C -glucose was always recovered as fatty acids. On the other hand, the difference between the amounts of ^{14}C fructose and ^{14}C -glucose recovered as fatty acids was influenced by the dietary history of the animals in that the difference was greatest (7 fold) in livers from fructose fed rats and least (2 fold) in livers from glucose fed animals (Table V). Thus, although glucose and fructose feeding increase the activities of fatty acid synthesizing enzymes to the same extent, each sugar had a selective enhancing effect on its own conversion to fatty acids in rat liver slices. This was a time dependent effect, suggesting that the selective enhancement of the fructose or glucose diet on the synthesis of fatty acids from fructose or glucose respectively was due to inductive changes in portions of the metabolic pathway proximal to the fatty acid synthesizing enzymes. The conclusion that the differential synthesis of fatty acids from fructose or glucose is based on events prior to the entry of substrate into the fatty acid synthesizing pathway was strengthened by the observation that no matter which products

Table VII. Comparison of enzyme activities for the glycolysis of fructose and glucose in human liver. Activities are the mean \pm S.E. of μ moles substrate metabolized per min per mg protein. The figure in parenthesis is the number of samples from different livers. Data from Zakim et al. (26)

Fructose enzymes	Activity	Glucose enzymes	Activity
Fructokinase	77.4 ± 3.6 (7)	Glucokinase	3.13 ± 0.31 (6)
F 1 P Aldolase	50.4 ± 3.8 (4)	Hexokinase	1.76 ± 0.25 (6)
Triokinase	34.5 (2)	Phosphofructokinase	30.5 ± 3.8 (7)
Alcohol dehydrogenase		F 1 6-P Aldolase	54.7 ± 7.0 (4)
NADH	65.3 ± 5.7 (7)	Pyruvate Kinase	22.1 ± 29.4 (2)
NADPH	48.2 ± 6 (6)		

reaction are at least as great as the capacity of the liver for phosphorylation of fructose. On the other hand, at saturating concentrations of glucose, the synthesis of glucose-6-P is still likely to be rate limiting for the overall glycolysis of glucose in the liver cell. Although it has recently been shown by Woods, Eggleston and Krebs (21) that fructose-1-P aldolase is inhibited competitively by DMP during fructose metabolism, thereby increasing large increases in the hepatic concentration of fructose-1-P it is still unlikely that the rate of conversion of fructose falls below that for glucose.

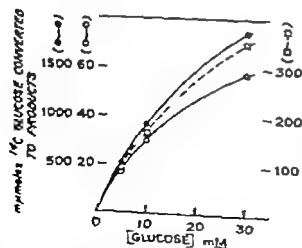


Fig. 3. Dependence of fatty acid synthesis by human liver slices on glucose concentration. Data is amounts of ^{14}C -glucose recovered as fatty acids (\circ), CO_2 (\bullet), and glyceride-glycerol (\square).

In addition to the low maximum activity for the phosphorylation of glucose, the hepatic enzyme primarily responsible for this phosphorylation, glucokinase, has a poor affinity for glucose, the K_m for glucose being approximately 10 mM (9). Thus, even during the active absorption of sugar glucokinase is likely to be less than one half saturated with substrate (blood sugar concentration of less than 100 mg per 100 ml). It follows that if in some way the slow rate of glycolysis of glucose is responsible for its relatively poor conversion to fatty acids in comparison to fructose, the conversion of glucose to fatty acids should be highly concentration dependent within the physiologic range of glucose concentrations. This in fact is the case as shown for human liver slices in Fig. 3. Increasing the medium concentration of ^{14}C -glucose from 5.0 mM (90 mg per 100 ml) to 30 mM increases the rate of conversion of ^{14}C -glucose to fatty acids, and, in addition, to glyceride-glycerol and CO_2 . It seems clear that the greater hepatic production of fatty acids from fructose than glucose is in some way dependent on the greater rate of glycolysis of the former sugar in comparison to the latter. Two possible mechanisms could contribute to this effect. Either a glycolytic or Krebs' cycle intermediate is important for the dynamic regulation of fatty acid synthesis or the synthesis of fatty acids is limited by the availability of substrates.

Effect of fructose on hepatic concentrations of glycolytic and Krebs cycle intermediates

As mentioned above, it is well known that the administration of fructose increases serum concentration of pyruvate and lactate. Studies in animals have confirmed that a chronic administration of fructose increases the steady state level of several glycolytic and Krebs cycle intermediates in liver (4, 21). Chronic administration of fructose, as for example by ad libitum feeding of diets containing large amounts of fructose is also associated with increased hepatic concentrations of several metabolic intermediates along the pathway from fructose to fatty acids, including acetyl-CoA (23). Two studies in intact rats have demonstrated that the concentrations of some intermediates may increase only transiently despite the continued availability of relatively large concentrations of fructose in the blood (8, 23). Because, however, of considerable uncertainty as to the exact mechanism for the dynamic regulation of the fatty acid synthesizing pathway more specifically the activity of acetyl-CoA carboxylase, the significance of the effects of fructose on the concentrations of metabolic intermediates is difficult to determine. It is important, nevertheless, to consider two intermediates, α -glycerophosphate and citrate, which have been postulated to be of importance for the regulation of fatty acid synthesis.

It has been proposed that fatty acid synthesis can be controlled by its production of acyl-CoA compounds, which inhibit acetyl-CoA carboxylase *in vitro* (see ref. 25 for a brief review of this problem). Since acyl-CoA derivatives react with α -glycerophosphate the concentration of this latter compound could modulate theoretically the rate of fatty acid synthesis by increasing or decreasing the removal of acyl-CoA. More recent information indicates that it is unlikely that acyl-CoA compounds are important physio-

logically for the regulation of acetyl-CoA carboxylase (25). On the other hand, modulation of the rate of esterification of acyl-CoA variations in the concentration of α -glycerophosphate could alter the ratio of bound and free CoA which is known to influence the rate of oxidation of pyruvate to acetyl-CoA. Since this is an intermediate step for the conversion of carbohydrate carbon to fatty acids, inhibition of pyruvate dehydrogenase might limit the synthesis of fatty acids. However there is no correlation between *in vivo* rates of esterification of fatty acids and the hepatic concentration of α -glycerophosphate (25) so the significance of the concentration of α -glycerophosphate for metabolic regulation is uncertain. In the absence of knowledge of the factors controlling the rate of esterification, or for that matter the details of the pathway from fatty acid synthetase bound acyl chain to complex lipids, it is impossible to state whether or not esterification can directly influence the rates of fatty acid synthesis in livers from fed animals. On the other hand, it is worth pointing out, as the data in Tables I to IV show that the amount of carbon metabolized to CO_2 or glyceride-glycerol is far in excess of that recovered as fatty acids with either glucose or fructose as substrate. Furthermore, since 4 moles of glucose are required for the synthesis of 1 mole of palmitate, there is sufficient carbon in 1 mole of glucose to potentially esterify the palmitate derived from 24 moles. In view of these considerations and the data cited above (Tables I to IV) it seems unlikely that the flux of carbon from glucose to α -glycerophosphate could limit the incorporation of glucose into fatty acids.

Citrate, the other metabolic intermediate which could effect the functional state of acetyl-CoA carboxylase *in vitro* is required *in vitro* for assay of acetyl-CoA carboxylase. Citrate acts by converting an inactive monomeric form of the enzyme to an enzymatically

Table VIII. Comparison of tissue substrate concentrations to K_m values for the conversion of citrate to malonyl-CoA

	Reaction	
	Citrate \rightarrow Acetyl-CoA	Acetyl CoA \rightarrow Malonyl-CoA
K_m for substrate	$5.6 \times 10^{-4}M$	$1.9 \times 10^{-3}M$
Tissue substrate concentration	$4 \times 10^{-4}M$	$4.3 \times 10^{-3}M$

active polymeric form. As with α -glycerophosphate, however the importance of this in vitro citrate requirement is uncertain in that the concentrations of citrate required for in vitro activation are far larger than in vivo citrate concentrations (25). Also it has recently been shown that acetyl-CoA carboxylase can be isolated directly in an active form from livers of fed animals (20).

On the basis of the above arguments and data α -glycerophosphate or citrate are not primary factors for altering the fatty acid synthesis in vivo. It is unable to conclude therefore, the reason for the enhanced conversion of fructose to fatty acids as compared to glucose is that the rate of hepatic fatty acid synthesis is limited by the availability of substrate in fed animals. Since fructose is metabolized to pyruvate and acetyl-CoA at a greater rate than glucose fructose feeding in-

creases hepatic fatty acid synthesis to a greater extent than glucose. Consideration of the relationships between the K_m 's of some fatty acid synthesizing enzymes and the in vivo concentrations of their substrates is compatible with this idea. Thus, the tissue concentrations of citrate and acetyl-CoA are close to the K_m for citrate cleavage enzyme and K_m for acetyl-CoA carboxylase (Table VIII). Since these substrates are not evenly distributed within the cell, their concentrations in the cytosol, the primary site of fatty acid synthesis, could be far less than their K_m 's and increases in the concentration of these substrates could effect large increases in the rate of fatty acid synthesis. The total magnitude of this effect is likely to be larger than one would expect from an inspection of Table VIII for as shown by Srere (18) the concentrations of enzymes in liver cells are large in comparison to the concentrations of their substrates.

Quantitative importance of hepatic fatty acid synthesis in man. Unfortunately there is no direct measure of the absolute rate of hepatic fatty acid synthesis in man or any other large mammal, and in order for the enhanced conversion of fructose to fatty acids in liver to be of physiologic importance it would have to be shown that human liver can synthesize fatty acids at a rate consistent with the magnitude of dietary induced changes in the concentration of plasma triglycerides. In an attempt to obtain an approximate value for maximal rates of hepatic fatty acid synthesis we measured acetyl-CoA carboxylase activity in samples of human liver obtained during elective abdominal surgery. The data in each of 4 patients are listed in Table IX. It may be of importance that although patient No. 3 appeared to be eating normally prior to surgery he had a partial gastric obstruction secondary to a duodenal ulcer and the low-hepatic acetyl-CoA carboxylase activity in this patient as compared

Table IX. Acetyl-CoA carboxylase activity in human liver. Activity is expressed as μ moles $H^{14}CO_2$ fixed per min per mg protein

Patient	Acetyl-CoA Carboxylase Activity
1	0.8
2	3.98
3	0.438
4	1.59
Mean	2.0*

have been related to a subtle nutritional deficiency

Based on an average acetyl-CoA carboxylase activity of 2.02 μ moles H^{14}CO_2 fixed/min/mg protein and a liver weight of 3 per cent body weight, one can calculate that the liver in a 70 kg man could synthesize maximally 1 g of triglyceride/hr. With an acetyl-CoA carboxylase activity of 3.98 μ moles H^{14}CO_2 fixed/min/mg this value could be as high as 2 g/hr. Since the turnover rate of very low density plasma triglycerides in men with normal plasma triglyceride concentrations is from 2.0 to 10.7 mg/kg/hr (17) the rate of *de novo* triglyceride synthesis in the liver could be large in comparison to the turnover rate of the plasma very low density lipoprotein triglycerides. Although it is not possible to state with certainty on the basis of the acetyl-CoA carboxylase activities alone what the actual rates of hepatic fatty acid synthesis are, it is reasonable to conclude that *de novo* synthesis of fatty acids in the human liver is a quantitatively significant factor in the turnover of plasma triglyceride fatty acids. Although an effect on hepatic fatty acid synthesis is only one of several possible ways in which fructose containing sugars could increase the plasma triglyceride concentration, the observed effects of different carbohydrate diets on the plasma lipid concentrations in man and animals is consistent with predictions derived from the data on the more rapid *de novo* conversion of fructose to hepatic fatty acids as compared to glucose.

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EFFECT ON SERUM LIPIDS OF METABOLIC LOAD AND FRUCTOSE

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Abstract. Though the measurement of the concentration of substances in the serum may provide misleading evidence for metabolic studies, nevertheless findings of changes in the levels of various serum lipids have a high degree of association with vascular disease. Sucrose or one of its constituent monosaccharides, fructose, has been found to have an influence on serum triglyceride concentration — the effect on the serum cholesterol level being minimal — when compared with glucose or its polymers.

After the acute ingestion of sucrose a less striking fall is found in the serum triglyceride level than after glucose ingestion. Experimental diets containing a large proportion of carbohydrate result in a level of triglyceride that is higher when the carbohydrate contained fructose than when it contained glucose. The effect of dietary fructose on the fasting level of serum triglyceride is modified by the sex of the consumer, the amount and type of fat accompanying the fructose, the type of protein in the diet, the frequency of intake, etc.

The few studies on long term ingestion of fructose suggest that the rise in triglyceride concentration following fructose ingestion falls.

There is some evidence that in vascular disease the fructose metabolism may be more disturbed than that of glucose.

The concentrations of various substances in the blood have long been used as indicators

of metabolic disarray or impending disease. As blood is relatively plentiful and easy to obtain it is not surprising that exhaustive studies have been made of its composition, especially in disorders that are common and for which prediction and consequent prevention are still largely unknown. In arterial disease the lesion in the wall is fatty and in the absence of an easy method to assess the cause and extent of this disease the lipid components of the blood have been examined. A close association has been found between the complications of arterial disease and both the serum cholesterol and triglyceride levels, but no direct relationship has been shown, and in fact it is not unknown for the coronary arteries to become blocked as a consequence of arterial disease and the serum levels of both cholesterol and triglyceride to be within normal limits at the time. Consequently the effect of diet on serum lipids may be of purely academic interest or it may have considerable clinical importance. It is not the level of lipid in the serum per se that matters it is the disability that may arise or be associated with this elevation in concentration that is important.

DIETARY SUCROSE/FRUCTOSE IN
HYPERLIPIDAEMIA

As was pointed out in 1961 (1) and subsequently confirmed in a classification of hyperlipidaemia (13) there are some patients who are carbohydrate-sensitive in that the fasting serum triglyceride concentration increases as the intake of carbohydrate increases. The incidence of this condition is not known, nor is it known whether the triglyceride level of these patients is more sensitive to sucrose/fructose than to glucose and its polymers.

Vascular disease is a complication associated with carbohydrate-induced hypertriglyceridaemia (13) and it has been found that patients with peripheral vascular disease have higher levels of serum fructose after ingesting sucrose than do controls (30). Several weeks after a myocardial infarct the amount of ^{14}C , from both glucose and fructose, incorporated into the serum triglycerides is much greater than in the controls (24). More the radiolabel from fructose was incorporated into the serum triglycerides than glucose. This was found to apply to both the glycerol and fatty acid moieties of the serum triglyceride (33).

CONCLUSIONS

Thus it seems clear that the fructose component of the diet can give rise to responses of lipid metabolism that are different from those found after consuming glucose. The response of the serum triglyceride concentration to dietary glucose seems to be unaffected by those many factors that affect the serum triglyceride response to fructose. Included in the factors that modify the dietary fructose/serum triglyceride relationship are the sex of the consumer, the type of dietary fat accompanying the fructose, the frequency of eating the fructose, and the source of amino nitrogen in the diet.

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EFFECTS OF DIETARY FRUCTOSE AND SUCROSE ON PLASMA TRIGLYCERIDE METABOLISM IN PATIENTS WITH ENDOGENOUS HYPERTRIGLYCERIDEMIA

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Abstract. Since fructose is now marketed as a nutrient study has been initiated to compare the effects of fructose, sucrose and starch to a conventional dietary intake level on the plasma triglyceride concentration and turnover in patients with endogenous hypertriglyceridemia. Preliminary results are presented here.

Fructose or sucrose in a daily amount of 75 to 100 grams were added to the diet in an isocaloric exchange for starch in twelve hyperglycemic patients under hospital conditions. Each dietary period lasted for 10 to 20 days and the triglyceride turnover was studied by endogenous glycerol labeling technique at the end of each period. Five of the patients had manifest diabetes managed by diet or oral antidiabetic drugs.

Plasma triglyceride response to fructose was variable and the average change from the level observed during fructose-free diet was not significant. However in nondiabetic subjects a definite rising trend of triglyceride was noticeable. Sucrose caused significant increase of triglyceride concentration above that measured during fructose and starch diets. Diabetics were not included into the sucrose group, however the fractional removal rate of plasma triglycerides was identical during all three diets but the triglyceride production rate showed a rising trend during fructose and, particularly during sucrose

diet as compared to starch. Because of the limited number of subjects studied so far these results do not justify any definite conclusions.

It is now generally recognized that high carbohydrate intake tends to elevate the plasma triglyceride concentration and among different carbohydrates sucrose and fructose show the highest hyperglycemic activity (for review see (24)). In fact, the effect of sucrose has been attributed to its fructose moiety and the increase of plasma triglyceride concentration is believed to result from a greater incorporation of exogenous fructose than of glucose into hepatic and plasma triglycerides (3, 23). The main part of the human dietary studies carried out on influences of either sucrose or fructose on plasma lipids have used the sugars in amounts that far exceed those usually contained in the average diet of adult man (2, 4, 11—14, 19). In these experiments all dietary carbohydrate has been either starch or simple sugar and this has resulted in the very unusual daily sugar intakes of 200 to 500 grams. In one

study using more physiological amounts of sugars no difference was found in serum lipid levels on exchanging starch and sucrose in normoglyceridemic subjects (9). Comparison between the effects of dietary sucrose and fructose has previously been carried out in only one human experiment (12, 13).

Thus, in spite of a considerable number of dietary studies it is not yet clear whether the sugar intake at an average customary level has any influence on plasma triglyceride concentration in normal subjects or in patients with familial hypertriglyceridemia. The present study was undertaken to determine the plasma triglyceride response of hyperglyceridemic patients to interchanges of dietary sucrose, fructose and starch. Furthermore, the effect of the sugars on triglyceride transport kinetics was assessed in these experiments. This paper records the preliminary data of a more extensive study still in progress.

MATERIAL AND METHODS

Twelve subjects with endogenous type IV hypertriglyceridemia are included in this report. Four were women aged 59 to 62 years and eight were men ranging from 26 to 67 years. Five patients had manifest diabetes which was either untreated or controlled by oral antidiabetic drugs. This drug treatment was not changed during the dietary experiments. No drugs influencing directly the lipid metabolism were used either before or during the study. The patients had been originally detected because of symptoms of coronary heart disease, obesity or diabetes but at the time of the study none had had a recent coronary event.

All subjects were hospitalized and put on an approximately isocaloric mixed hospital diet. The experimental period was started when the plasma triglyceride concentration had stabilized to certain level, which in most instances was much less than the value on admission. Thus, the home diet was qualitatively or quantitatively more hyperglyceridemic than the standard hospital regimen. The fall of plasma triglyceride might possibly be ascribed to the absence of alcohol in the hospital. Many of the patients had certainly consumed it under home conditions even though none was known to be alcoholic.

The actual experiment consisted of two or three periods (designated 0, F and S) during each of which the patients were given isocaloric diet containing 45 per cent of calories as carbohydrate (CHO), 33 per cent as fat (mainly saturated) and 20 per cent as protein. The only difference between the periods was in the quality of dietary carbohydrate. During Period 0 the main carbohydrate was starch, which provided an average of 80 per cent of CHO calories. The rest constituted of approximately 25 grams of lactose and 20 grams of sucrose daily. In the diet of Period F 75 or 80 grams/day of starch was exchanged for the same amount of fructose added in the basal diet as free substance (not incorporated into the foods). During the Period S starch was substituted for 75 or 80 grams of sucrose the daily consumption of which thus amounted to approximately 100 gram corresponding to 20 to 25 per cent of total calory supply. The length of each dietary period varied between 10 and 20 days and their sequence was alternated in a random fashion. In patients with manifest diabetes the Period S was omitted and comparison was only made between fructose-containing and low-sugar diets while in the nondiabetic subjects all three regimens were instituted. Weight was recorded every day and if systematic deviations were noticed the calory content of the diet was increased or decreased respectively without changing the relative amounts of the three main constituents.

Plasma triglyceride concentration was recorded every second day (27) and at the end of each period plasma triglyceride turnover was determined by the endogenous ^3H -glycerol labeling technique (10) the details of which have been described in a previous paper (25). The slope of the plasma triglyceride- ^3H radioactivity decay curve was analysed by a digital computer to obtain the best fit for fractional rate constant, k . From this parameter the total turnover (production) rate V of endogenous plasma triglycerides was calculated by using the formula

$$V = k \times 0.45 \times S \times B \quad (\text{mg/kg/hr})$$

where S is the mean plasma triglyceride concentration (mg/100 ml) during the test (four determinations in 8 hours) the factor 0.45 is derived from the assumption that plasma space represents the distribution volume of circulating triglycerides and forms 45 per cent of body weight. The factor b is a correction for the relative decrease of plasma volume on increase of relative body weight (1).

RESULTS

The effect of interchange of starch and fructose was studied in ten subjects. The triglyceride transport data are presented in Table I, which shows that plasma triglyceride concentration, fractional transport rate and production rate were closely similar during the two dietary periods. The corresponding values for five nondiabetic subjects passing through all three experimental periods are demonstrated in Table II. It is seen that during the sucrose period both triglyceride concentration and production rate showed a rising trend as compared to the values during the other two diets but because of the small number of cases none of the differences was significant at 0.05 level. The individual responses of plasma triglyceride concentration to the three diets appear from Fig. 1 which includes two additional

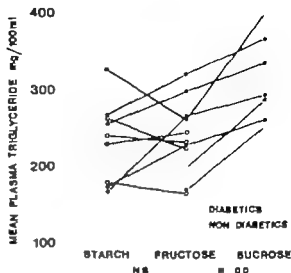


Fig. 1 Mean plasma triglyceride concentration during three dietary periods. Fructose and sucrose were administered 75 or 80 grams per day in an isocaloric exchange for starch. Analyses were made every second day and thus the recorded values are averages from 8 to 9 individual measurements.

Table I. Plasma triglyceride concentration and turnover (mean \pm s.d.) during starch and fructose diets

Diet	TG concentration	TG fractional turnover	TG production rate
n = 10	mg/100 ml	h ⁻¹	mg/h/kg
Starch	206 \pm 185	0.167 \pm 0.070	14.3 \pm 4.0
Fructose	236 \pm 119	0.163 \pm 0.062	14.4 \pm 4.5

Table II. Plasma triglyceride concentration and turnover (mean \pm s.d.) rate in nondiabetic hypertriglyceridemic subjects during three dietary periods

Diet	TG concentration	TG fractional turnover	TG production rate
n = 5	mg/100 ml	h ⁻¹	mg/h/kg
Starch	230 \pm 48	0.180 \pm 0.041	14.1 \pm 2.1
Fructose	239 \pm 36	0.172 \pm 0.020	14.4 \pm 2.3
Sucrose	270 \pm 93	0.179 \pm 0.044	16.8 \pm 4.5

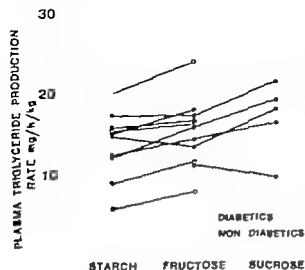


Fig. 2 Plasma triglyceride production (turnover) rate at the end of three dietary periods each lasting for 10 to 20 days.

subjects where fructose and sucrose were compared but the data of starch period had to

omitted because the weight did not reach a steady-state level. The response to fructose was highly variable but it seems that most of the hyperglyceridemic patients without diabetes showed a definite rising trend of their plasma triglyceride level when transferred from starch to fructose diet. On the other hand, an increase of plasma triglyceride occurred in every subject when starch or fructose were exchanged for sucrose. With this number of cases the differences sucrose fructose and sucrose starch are not significant. Plasma triglyceride production rate in individual cases is illustrated in Fig. 2. A slight trend to increase is apparent when fructose is compared with starch and when the sucrose period is compared with the non-sucrose ones but, again, there is no statistically significant difference in the triglyceride synthesis between the three periods.

DISCUSSION

The dietary sugar intake shows great individual and local variations but it usually ranges from 50 to 150 grams per day corresponding to 10 to 25 per cent of total calories (34). All those human studies where the hyperglyceridemic effect of sucrose has been demonstrated have employed more or less unphysiological diets with sugar covering 40 to 100 per cent of calories and amounting to 200 to 500 grams daily (2, 4, 11-14, 19). With smaller sucrose intakes no effect on plasma triglyceride level has been observed in normoglyceridemic subjects (15) but in patients with type III or IV hyperlipoproteinemia Little et al. (1970) reported an increase of plasma triglyceride level when 20 per cent of calories was given as sucrose instead of starch. Low-sucrose and average sucrose (85 g/day) diets were compared in human volunteers by Mann et al. (20) who found significantly lower triglyceride levels with the former regimen. However the decrease coincided with weight loss and this makes the evidence for sucrose inconclusive.

The present study demonstrates that in subjects with primary endogenous hyperglyceridemia sucrose intake at an average level of 100 grams daily tends to increase plasma triglyceride concentration from the value present at low sugar (about 20 g/day) consumption. Under the controlled dietary conditions used here the increment remained relatively slight but on the other hand, no experience was gained on the behaviour of plasma triglyceride under a more prolonged feeding of sucrose at this intake level. It is known from earlier studies that the effect of high sucrose diet on serum lipids is dependent on age, sex and nature of dietary fat. Thus hyperglyceridemia is not induced by sucrose in premenopausal women (16) or in subjects eating much polyunsaturated fat (3). In these respects the patients of the present study should have been particularly sensitive

in sucrose since all were either men or post menopausal women and their dietary fat was mainly saturated. The resistance of premenopausal women to any hyperglyceridemic factor may be accounted for by their effective plasma triglyceride removal system (25).

As fructose is now being marketed at a reasonable price and it is advertised as a sweetener to replace sucrose, a comparison between these two sugars with regard to their effect on plasma triglyceride metabolism has become important. It is known that fructose readily induces hypertriglyceridemia in rats (26) and in man (13, 17). In the study by Kaufmann and associates (13) fructose appeared to be slightly more hyperglyceridemic than sucrose. In the present series, on the other hand, fructose seemed to be slightly less active than sucrose in this respect but the number of subjects studied is too small and the differences are too slight to allow drawing of any definite conclusions at this stage of the study. The present results also suggested that well controlled diabetics can tolerate a substitution of 80 grams of fructose for starch daily without any harmful effects on either blood glucose or plasma triglyceride. The possible effect of an equal amount of sucrose on the control of diabetes and on plasma lipid levels in diabetic patients is not known, however.

The metabolic effects of dietary starch, sucrose and fructose show differences which make it possible to explain the different actions of these carbohydrates on plasma triglyceride transport. The present kinetic data indicate that the fractional removal rate is not influenced by the nature of dietary carbohydrate and this rules out the possibility that the sugars elevate plasma triglyceride by interfering with the triglyceride elimination from blood stream. The changes observed in plasma triglyceride concentration are therefore wholly accounted for by corresponding alterations in the rate of triglyceride secretion into plasma. It seems that

this rate is lowest with starch diet and is increased with fructose but even more with sucrose. That fructose increases the plasma triglyceride concentration more than an equivalent amount of starch might be accounted for by the markedly greater hepatic uptake of fructose than glucose (22, 31) and by the increase of glycerol phosphate content of the liver after fructose load (6, 35). Of orally administered fructose a much higher proportion is converted to hepatic and plasma lipids than of dietary glucose (or starch) (18, 21, 23) and a similar difference in the incorporation of carbon from the two sugars into hepatic lipids is observed also in vitro (23, 28, 36). A more extensive discussion on the possible explanations for these differences between starch and fructose (or sucrose) has been presented elsewhere (24).

The more marked stimulating effect of sucrose than of fructose on hepatic production of plasma triglycerides might be accounted for by the different responses of blood glucose, plasma insulin and FFA to sucrose and fructose. These three factors are currently believed to be important stimulators of plasma triglyceride synthesis (for references see 24). After sucrose load the magnitude of hyperglycemia, hyperinsulinemia and rebound increase of FFA are all more pronounced than after ingestion of similar amount of fructose (22). In the concentrations reached in peripheral blood after oral loads fructose does not discharge insulin from β cells (7, 8, 30) and as a consequence of the absent hyperinsulinism no hypoglycemia and concomitant rise of plasma FFA follows intake of fructose as occurs after sucrose. On the other hand, fructose itself inhibits the liberation of FFA from adipose tissue as effectively as glucose (29). In sucrose the metabolic effects of glucose and fructose are combined and this is particularly favorable for enhancement of plasma triglyceride synthesis. Thus, sucrose not only causes a rapid increase of the blood glucose and plasma insulin levels but simultaneously an excess of glycerol

phosphate is formed in the liver. That all these factors effectively promote the synthesis and release of triglycerides by the liver is shown by the experiments of Topping and Mayes (33) where both fructose and insulin separately stimulate production of very low density lipoproteins by rat liver perfused with a high glucose medium.

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THE SIGNIFICANCE OF SUCROSE IN PRODUCTION OF HYPERTRIGLYCERIDEMIA

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Abstract The relationship between endogenous and carbohydrate-induced hypertriglyceridemia (C-HTG) is reviewed. Different effects of sucrose, glucose, fructose, and starch on serum lipid levels in patients with C-HTG are demonstrated. The significance of these effects in patients with C-HTG and their relation to those in normal subjects is discussed.

There is much confusion as to the proper use of the term carbohydrate-induced lipemia. As originally defined by Ahrens et al. (2) it referred to those patients in whom feeding of a diet rich in carbohydrates resulted in an increase of serum triglycerides (S-TG). However S-TG levels of normal subjects may respond, at least temporarily to dietary carbohydrates in a similar fashion (1, 9, 24, 32). In addition, in patients with hypertriglyceridemia (HTG), elevated S-TG levels are found on a regular diet, in which carbohydrates amount to only 40—50 per cent of the total calories, while on the same diet in normal subjects normal S-TG levels are found. Therefore, the term endogenous hypertriglyceridemia has been suggested for these

patients (14, 33). Endogenous HTG indicates that the elevated S-TG are derived from de-novo synthesis, mainly by the liver. On the other hand, in exogenous HTG the elevated S-TG are derived directly from dietary fat. The term endogenous S-TG does not, however differentiate between S-TG originating from re-esterification of the free fatty acids liberated from adipose tissue by lipolysis and S-TG derived from de-novo synthesis of fatty acids from carbohydrates and other precursors. Thus, fatty acids for the synthesis of S-TG are not necessarily derived from carbohydrates, and, therefore, not all patients with endogenous HTG are necessarily carbohydrate-induced. However all patients with carbohydrate-induced hypertriglyceridemia (C-HTG) belong by definition to the group of endogenous HTG.

The classification of Fredrickson et al. (21) is not relevant to the above considerations. This classification, which is now used by almost all investigators and clinicians, refers to the kind of lipoprotein which transports the excessive amounts of serum lipids. However since Fredrickson's Type III and IV refer to increased levels of very low density lipo-

Table I. The effect of a carbohydrate rich diet and a low-calorie diet on serum lipid levels in a patient with hypertriglyceridemia

Diet	Weight (Kg)	Serum triglycerides (mg/100 ml)	Serum total cholesterol (mg/100 ml)
Ad lib.	74.0	2710	632
Carbohydrate-rich ¹	74.0-72.5	664	339
Fat rich ¹	72.5	493	332
Ad lib.	68.8	3180	740
Low-calorie diet ²	66.8-63.8	169	227

¹ Diets were administered for 4 weeks. Carbohydrates comprised 75% (mainly as starch) and fats 45% (mainly as vegetable oil) of total calories. S-TG and TC values are averages of the whole period.

² Diet consisted of 1000 calories (30% carbohydrates and 30% fat) and was administered for 7 weeks. S-TG and TC values are averages of the last 3 weeks.

proteins (VLDL) or to the appearance of an abnormal lipoprotein in Type III these types are included in endogenous HTG as well. Indeed, Glueck et al. (22) found only half of their patients with Type IV hyperlipemia to be carbohydrate induced. As to Type V in which an elevation of both VLDL and chylomicrons is found, at least part of the increase in S-TG is of endogenous origin.

The term endogenous HTG should therefore be applied to all patients with increased VLDL, normal or abnormal, regardless of the composition of the diet consumed. Abnormal insulin secretion (14-16) obesity (5) and alcoholism (28) may be the most important etiological factors. However disturbances of S-TG removal, as found in insulin deprivation (10) or on administration of certain drugs as steroids (11) are also included in the group of endogenous HTG. On the other hand, C-HTG is a special case of endogenous HTG and includes patients in whom feeding of a high carbohydrate diet of more than 65 per cent of total calories, raises S-TG levels above those found in normal subjects on a similar diet. C-HTG also includes patients with endogenous HTG in whom isocaloric restriction of carbohydrates to less than 40-50 per cent of total calories with no concomitant weight loss, results in a reduction of S-TG

levels to below those found when the patients were on a normal diet. In this latter case S-TG levels need not necessarily be reduced to normal by carbohydrate restriction. In other words, endogenous HTG refers to the de-novo synthesis of excess S-TG regardless of the composition of the diet. The term C-HTG applies to those patients in whom the de-novo synthesis of excess S-TG is a result of the amount of carbohydrates in their diet.

As an example of this approach the following case is presented. In a 55 year old moderately obese woman, S-TG level of 2710 mg/100 ml and total cholesterol (TC) of 632 mg/100 ml were found on routine examination, and on an ad lib diet. She was put on a diet of 75 per cent of calories from carbohydrates, however most of the carbohydrates were given as polysaccharides. Her S-TG decreased to 664 mg/100 ml and TC to 339 mg/100 ml. When fats were substituted isocalorically for carbohydrates a further decrease of S-TG occurred (Table I). After some years the patient was reexamined and again high lipid levels were found. This time she was treated for her obesity with a 1000 calorie reducing diet (30 per cent of calories as carbohydrates, 30 per cent as fats and 40

per cent as proteins). She lost about 30 kg and again S-TG and TC decreased. Thus in this patient by the latter treatment obesity was proven to be an important factor in maintaining her endogenous HTG. However even without restriction of caloric intake and resulting weight loss, carbohydrate inducibility of HTG could be demonstrated.

The production of HTG by dietary carbohydrates in C-HTG is not only dependent on their amount in the diet but on their quality as well (28, 29). In many patients with C-HTG isocaloric substitution of sucrose for starch, usually without significant weight gain, caused a further increase of S-TG and TC. However in most cases S-TG and TC levels were also elevated on the starch diet (Table II). In one patient (L. J.) elevated S-TG were found on the sucrose diet only while in another patient (W. M.) there was no change in S-TG levels when sucrose was substituted for starch. In this latter patient, though, feeding of fructose resulted in a marked elevation of S-TG.

The effect of sucrose in increasing serum lipid levels especially TG is not peculiar to patients with C-HTG. It is also found in normal subjects (3 @ 7, 41) especially in men and postmenopausal women (13, 35, 36, 37). However this effect cannot be demonstrated in either all normal subjects (32) or in all patients with C-HTG (27). On the other hand, in many patients with C-HTG this difference between the response of serum lipids to starch versus sucrose was much more pronounced than in normal subjects, and differences of S-TG levels between the two diets up to 900 mg/100 ml were found.

Feeding of fructose, but not of glucose, led to an increase of serum lipids in normal subjects similar to that following sucrose feeding (38) and more labelled fructose than glucose was incorporated into S-TG (40). Patients with C-HTG also responded to prolonged feeding of fructose with increases in S-TG levels and usually also of TC. The effect of fructose in raising S-TG was sometimes much more pronounced than that of

Table II. Comparison of the effect of dietary starch and sucrose on serum lipids.

Patient	Days		S-TG (mg/100 ml \pm S.D.)		TC (mg/100 ml \pm S.D.)	
	Starch	Sucrose	Starch	Sucrose	Starch	Sucrose
G.R.	36	28	905 \pm 198	1479 \pm 872	373 \pm 41	483 \pm 76
V.B.	18	20	805 \pm 112	1332 \pm 472	392 \pm 48	339 \pm 112
G.I.	11	9	722 \pm 66	1426 \pm 202	402 \pm 25	342 \pm 41
S.C.	24	14	664 \pm 170	1007 \pm 222	374 \pm 67	402 \pm 23
K.N.	23	25	822 \pm 167	949 \pm 214	376 \pm 20	352 \pm 89
P.R.	29	14	282 \pm 62	1184 \pm 282	212 \pm 28	278 \pm 25
L.J.	28	14	168 \pm 34	286 \pm 112	197 \pm 13	225 \pm 17
W.M.	21	35	234 \pm 68	282 \pm 42	186 \pm 21	216 \pm 14

¹ Difference significant: t 0.05 $> p > 0.01$

² Difference significant: t $p < 0.01$.

S-TG = Serum triglycerides TC = Total cholesterol.

Values are averages for the whole period. Carbohydrate content of diets 75-80 % of calories. Content of sucrose in sucrose diet, 68 % of carbohydrates.

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CONCLUDING REMARKS

CONCLUDING REMARKS

E. R. Froesch

Metabolic Unit, Department of Medicine University of Zurich, Switzerland

First of all I wish to thank the scientific committee and in particular Dr Nikkilä and Dr Huttunen for the invitation to participate in this very successful symposium. My thanks in the name of all participants go also to the Endocrine Society as the organizer of this symposium. I have long tried to figure why an endocrine society may want to organize a symposium on fructose. This problem puzzles me particularly because I am right now president of the Swiss Society of Endocrinology and since I also have a major interest in fructose. I venture to give an explanation. It must have to do with the sperm. The sperm belongs to the domain of the endocrinologist. Sperms do feed mainly on fructose and, as it seems, successfully and without any harm to their lipid metabolism. We may say without any exaggeration that none of us would be here if fructose had not been with us until conception.

Before going any further I would like to extend my thanks to the sponsor of the symposium, the Finnish Sugar Company which has provided so many goodies to make us feel happy and at home. They have suc-

cessfully and convincingly demonstrated that nothing not even fructose, can substitute a well prepared, excellent dish of fish or meat, smoked or cooked.

Fructose is a very early acquaintance of men since he is incubated in a fructose containing medium already as a sperm. The fetus then loses the habit of consuming fructose. The newborn may again get in touch with it 1 or 2 days after birth. Most newborns tolerate sucrose and fructose very well. However a few among them are killed by fructose. Dr Perheentupa and Dr Schapira have reported on hereditary defects of fructose metabolizing enzymes, the most frequent of which is hereditary fructose intolerance. Children with hereditary fructose intolerance may die within 1—2 months after birth if fructose or sucrose is present in their formula. Fructose does not harm children with fructokinase deficiency. Also, patients with essential fructosuria do not present an increased incidence of atheromatosis. Thus, increased blood levels of fructose by themselves do not harm blood vessels. It is of interest that only a few years

attention has focussed on the possibility that endogenously formed fructose and sorbitol may be causally related to atheromatosis. Aldosereductase and sorbitol dehydrogenase which convert glucose to sorbitol and fructose are present not only in the prostate and seminal vesicles to prepare the nutritional provision for the sperm for its journey to the ovum. Here these enzymes play an important role in reproduction. Their presence in the arterial and arteriolar wall, however may be harmful. At high blood sugar concentrations, these enzymes convert appreciable quantities of glucose to sorbitol and fructose. Sorbitol is trapped in these cells since there is no membrane carrier for this polyol. Sorbitol increases the intracellular osmolality leads to water uptake by the cell and to changes of the shape of cells and of the contacts between them. It is conceivable that swollen endothelial cells may loose touch so that lipoproteins may penetrate between cells and get trapped in the arterial

1 Diabetic arteriopathy may therefore, pathogenetically related to the endogenous of fructose and sorbitol from glucose. Thus, it is possible that the enzyme system which makes life possible by providing fuel for the sperm kills men later on by promoting atheromatosis.

Let us return quickly to inborn errors of fructose metabolism. A new hereditary disease fructose-1,6-diphosphatase deficiency has been described by Baker and Winegrad. These children have frequent attacks of acetonemic vomiting in combination with hypoglycemia and most of them die in early infancy. These children maintain normoglycemia during fasting as long as hepatic glycogen can be broken down and released by the liver as glucose. Once the glycogen stores are exhausted glucose can no longer be produced since fructose-1,6-diphosphate cannot be converted to fructose-6-phosphate due to the deficiency in fructose-1,6-diphosphatase. Fructose leads to acute hypoglycemia, but in contrast to hereditary fructose

intolerance of fructose U¹⁴C (20 mg/rat) in the presence (10 mU/rat) and absence (ant. insulin serum) of insulin (n = 7)

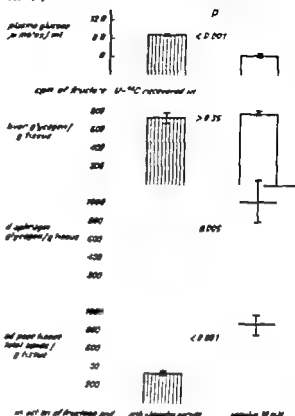


Fig. 1 Rats weighing 100 g were anesthetized with Valium® 20 mg of fructose with 1 μ Cl of U¹⁴C fructose was administered intravenously together with anti-insulin serum or insulin. The rats were decapitated 30 minutes after the intravenous injection and the blood and tissues were analyzed for carbon-14. (From Froesch et al., Europ J clin. Invest 2 8, 1971)

intolerance without nausea and vomiting. Therefore, these children do not develop a distaste for sweets. Fructose induced hypoglycemia is due to an inhibition of phosphorylase by fructose-1 phosphate and fructose-1,6-diphosphate at low intracellular concentrations of inorganic phosphorus.

I would like to put forward a plea to the manufacturers of baby food. Hereditary fructose intolerance is a frequent inborn error of metabolism. Many newborns with hereditary fructose intolerance are still being

Metabolism of ^{14}C sorbitol ($100\text{ mg}/100\text{ g}$) in the presence (insulin) and absence (anti-insulin serum) of insulin (n 10)

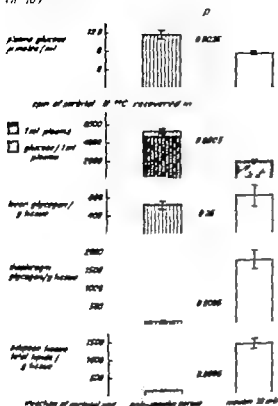


Fig. 2. Similar experiment as that shown in Figure 1 with sorbitol instead of fructose (From Froesch et al. *Europ. J. clin. Invest.* 2, 8, 1971)

killed by fructose in the first few months of their life. The diagnosis of hereditary fructose intolerance is difficult in newborns receiving fructose in their formula from birth on. Therefore, pediatricians should forcefully convey the idea to food manufacturers to make every effort to replace sucrose and fructose by glucose or maltose in baby food.

We have heard nothing about the use of fructose in parenteral nutrition. In many countries fructose is used as the main carbohydrate during and after surgery. The reasoning behind this therapy is the notion that fructose is metabolized in an insulin-independent fashion. This notion is based on a misunderstanding. It is true that the

half-life of fructose is short, i.e. approximately 3 minutes and that it is the same in diabetics as in normal subjects. We have illustrated during this symposium that fructose is taken up and metabolized by the liver. What is its fate? Oxidative processes in the liver are geared by the synthetic and detoxication functions and not by substrate availability. The latter determines only whether the glucose, lactate, free fatty acids or in the case of fructose infusion, fructose is used as the major fuel. Since the half-life of fructose is so short, only a minimal portion of an intravenous load will be oxidized directly. Some fructose will be stored as glycogen and later released by the liver as glucose and some will be incorporated into total lipids. However, the greatest portion of an acute fructose load is rapidly converted to glucose and released into the blood. Fructose conversion to glucose is readily detectable in an insulin-deficient diabetic whose blood sugar increases rapidly after a fructose load. In normal subjects hyperglycemia after fructose does not occur due to compensatory insulin secretion. I wish to illustrate this in a slide (Fig. 1). This experiment was carried out with normal rats which received a load of fructose together with fructose- ^{14}C . One group was simultaneously injected with anti-insulin serum, whereas the other group was treated with insulin. What are the essential differences between the two groups attributable to the presence or absence of insulin? Plasma glucose is raised in the rats receiving anti-insulin serum. Incorporation of fructose- ^{14}C into liver glycogen is the same in both groups. The difference of plasma glucose can be accounted for by the effect of insulin on the incorporation of glucose- ^{14}C originating in the liver from fructose- ^{14}C into muscle and adipose tissue.

The next slide shows a similar experiment, this time carried out with sorbitol- ^{14}C instead of fructose (Fig. 2). The counts in the glucosazones and in plasma were also deter-

quantities of fructose I do not think that this very important question has been answered during this symposium to the satisfaction of all of us.

Future research will tell us whether fructose is really the crucial nutriment to make us feel that life is so sweet on the sunny side of the street

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Acta Medica Scandinavica

Supplementum 543

Family Studies in Systemic Lupus Erythematosus

By Rolf A. Larsen

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**Family Studies in
Systemic Lupus Erythematosus**

Family Studies in Systemic Lupus Erythematosus

BY
ROLF A. LARSEN

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The papers "Family studies in systemic lupus erythematosus (SLE)
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Oslo, January 1972.

Rolf A. Larsen

Introduction

During the first half of this century at least 40 reports of familial discoid lupus erythematosus (DLE) appeared in the literature (for references, see Leonhardt (8)). Klemperer et al. (2) in their autopsy protocol, noted a family in which the mother had DLE and the daughter autopsy-proven systemic lupus erythematosus (SLE) (cited by Danbolt (2)).

SLE in identical twins was reported by Davis & Gutridge (1) but during the last twenty years only a few new reports of SLE in twins have been published. Until 1966 there were 46 complete and incomplete reports of familial SLE (for references, see Leonhardt (1)). Mass (9)).

Pedigree analyses around patients with SLE, performed by Leonhardt (7) and Larsson & Leonhardt (6) showed familial aggregation of hypergammaglobulinemia. Hypergammaglobulinemia was shown to precede SLE, and this familial abnormality seemed to be an indication that SLE developed in genetically predisposed individuals. The familial aggregation of hypergammaglobulinemia was confirmed in more recent studies (for references, see Mass (9)). Most studies showed increased frequencies of antinuclear factors (ANF), rheumatoid factors (RF), hypergammaglobulinemia, and rheumatic diseases in relatives of patients with SLE compared with controls, and multiple aberrations were shown in some families. Mortuo et al. (10) demonstrated that three-quarters of their 19 families had at least one first degree relative with at least one abnormality. Poffek (11) found increased frequencies of ANF both in first and second degree relatives of 43 patients with SLE, and the occurrence of familial SLE increased the frequency of ANF in the families.

Most studies showed a wide spectrum of rheumatic diseases, but ANF, RF and hypergammaglobulinemia were also present in sera of apparently healthy family members. Sigvald et al. (12) carried out a systematic study in a defined population with proper controls, using index-case and index-control families and found increased frequency of hypergammaglobulinemia in the families of SLE probands. Leonhardt (8) studied index-case families in a defined area in Sweden, using the spouses of the relatives of the SLE probands as controls, and demonstrated statistically significant differences between the relatives and the controls for several variables.

My family studies in SLE and SLE-like syndromes started about 10 years ago when I first presented pedigree analysis including 37 members, around a forty-year-old woman with Hodgkin disease and symptoms resembling SLE (Larsson (3)). Pedigree analyses around SLE-like syndromes were reported in Lund (Larsson (4)) and both reports demonstrated aggregation of hypergammaglobulinemia and a variety of autoimmune disorders in the family members. A third report (Larsson (5)) including 15 first and second degree relatives around a patient with classical SLE, revealed that almost all members had one or more clinical and serological abnormalities. The serum of the mother of the SLE proband revealed antinuclear factors, including LE factor, rheumatoid factors, thyroid antibodies and hypergammaglobulinemia, but she was apparently healthy.

Systematic and extensive index-case family studies in SLE — with the spouses of the relatives and the SLE probands used as controls — were started in 1963 and the main purposes were

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Systematic and extensive index-case family studies in SLE — with the spouses of the relatives and the SLE probands used as controls — were started in 1963 and the main purposes were

- a) to study clinical and serological aberrations in relatives of patients with SLE, and if familial aggregation were present to characterize the distribution in defined groups of relatives, including both first and second degree relatives,
- b) to discover whether the aberrations in the relatives were related to the clinical findings in the SLE probands by classifying the patients with SLE according to the syndromes they exhibited during active stages of the disease
- c) to investigate whether the aberrations in the relatives had a genetic basis.

The results of my studies are described in the 9 original papers listed in the Contents. An Editorial in the *Journal of Chronic Diseases* summarizes the results and presents a general discussion.

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I A proband material from central eastern Norway

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ABSTRACT

A survey of SLE in an area from central eastern Norway is given, together with clinical and laboratory data on the 72 patients diagnosed as SLE in this area during the years 1959-1963. The main proportion of these cases would have been classified as definite SLE according to diagnostic criteria given in the literature. Annual incidence and prevalence rates were lower than rates presented from other countries. The frequency of various clinical manifestations is consistent with previous reports. The SLE patients were classified according to syndromes they exhibited during the active stages of their disease, and the manifestations in various syndromes were compared.

The SLE material serves as proband material for family studies, and sub-grouping of SLE cases is the basis for comparison of the findings in relatives of these various groups of SLE probands.

Since 1828 the concept of systemic lupus erythematosus (SLE) as a disseminated disease has gradually developed (for references see 26)

It has not yet been possible to recommend definite diagnostic criteria for SLE (20). The need for such criteria is urgent in the study of the epidemiology and the heredity of the disease. However SLE cases may be grouped according to the syndromes they exhibit during active disease (1).

The main purpose of the present paper is to describe a group of patients with SLE from central eastern Norway as basis for discussion of the abnormal clinical and serological findings in the families of the patients.

METHODS AND CRITERIA

Material. The patients with SLE were collected from 6 fylker (shires) Akershus, Buskerud, Hedmark, Oslo, Vestfold and Østfold, in central eastern Norway with an area of 54,036 km² and population of 1.43 million (4). About 54 per cent of the population lived in rural districts. Central eastern Norway has an entirely caucasoid population, with very stable residence.

All hospitals within the area were visited by the author who examined the patient registers for the years 1955-1965. The clinical and autopsy records of patients with diagnosis of

SLE (including suspected cases) and other collagen diseases were critically reviewed, and all LE cell preparations were re-examined. The patients were generally readmitted to the same hospital during the period. Patients admitted to two or more hospitals inside the area were examined for change in diagnosis. Suspected SLE cases were followed up for later admission to their hospitals. Patients with definite SLE diagnosed prior to 1959 were included in the material, if they were still alive after 1959. Fifty-one (70.4 per cent) of the 72 patients accepted as probands with definite SLE were personally examined by the author.

All available data relevant for diagnosis of SLE according to the literature were considered. Combinations of three or more of the following criteria were crucial for the diagnosis of SLE: intermittent fever, polyserositis, myocarditis, nephritis, pulmonary lesions, lupus skin lesions, skin hemorrhages, numerous LE cells, thrombocytopenia, leucopenia and hypergammaglobulinemia. However numerous LE cells (at least 15-20 LE cells per slide) was most often decisive. The patients were excluded mainly on the grounds of negative LE cell preparations. Negative autopsy findings in patients with history otherwise typical of SLE did not exclude diagnosis of SLE, nor did negative LE cell preparations, if necropsies were consistent with SLE (8). The syndromes which

are usually not considered as being typical for SLE, such as lupoid hepatitis, rheumatoid arthritis with LE cells and few systemic manifestations, or drug induced SLE, were excluded. Generally clinical manifestations alone did not sustain the SLE diagnosis. Thus the present SLE material consists of 4 different groups of patients: patients with classical SLE including numerous LE cells; patients with symptoms compatible with both SLE and rheumatoid arthritis including positive LE cell tests, patients with SLE and chronic discoid skin lesions; and lastly patients with clinical and pathological SLE manifestations (wire loops, periarterial fibrosis of the spleen or hematocytin bodies) but with negative LE cell preparations.

The severity of SLE makes it likely that the patients in some time during the disease had to be hospitalized (73) but some benign cases of SLE are probably not admitted to hospital. Thus one male case was diagnosed by the author during his family studies, he had a titre of 1/8 in the indirect LE factor test (wide infra) transient nephritis, serositis and fever and responded well to corticosteroid therapy. Generally the diagnosis of SLE was made in the hospital in those cases when the patients exhibited systemic manifestations, but a few patients would not fit the present study primarily if the diagnosis of SLE or other collagen disease was not taken into consideration in hospitals; secondly if they lacked sufficient laboratory data. The facilities for hematological and histological investigations varied in the hospitals.

Blood specimens for LE factor determination has routinely been sent to the author from hospital inside the area after 1964 but additional distant case from the period 1959-1961 was discovered from this material.

A total of 165 different diagnostic criteria were summed up from nine papers (6, 9, 13, 16, 19, 21, 25, 27) where varying numbers and weights of significance were attached to these criteria. In the probands with SLE were compared with and classified according to diagnosis. The criteria in the 9 papers (6, 9, 13, 16, 19, 21, 25, 27) would have been used to define SLE patient by the criteria given in these seven papers, 69 (75 per cent) of the criteria given in 3 of the papers (6, 9, 13) per cent by the criteria given in all 9 papers, 5 of the

nine SLE cases which did not fulfil the criteria in all six papers would have been excluded by the criteria in one paper (13) mainly due to different views concerning the significance of necropsy findings and the LE cell phenomenon. Only one case would have been refused simultaneously by the criteria given in two papers.

Statistical methods. The data were punched on data cards and processed in a CD 3300 computer at the University of Oslo, Norway. χ^2 calculations were performed when two subgroups of SLE patients were compared using 2×2 tables and one degree of freedom. A continuity correction was used (7). P-values below 0.05 were regarded as significant.

Criteria:

(a) *Hematological changes.* (1) Anemia: hemoglobin concentration below 11.0 g per cent. (2) Leucopenia: white blood cell count below 3000 per mm^3 . (3) Thrombocytopenia: blood platelets below 150 000 per mm^3 . (4) Numerous LE cells: at least 15-20 LE cells per slide using the direct techniques (19, 28). (5) Few LE cells: less than 15 LE cells per slide and extracellular material (ECM = LE bodies = globs) largely absent. (6) Few LE cells and ECM: less than 15 LE cells per slide but abundant ECM. (7) ECM/ECM present but LE cells absent. (8) Positive Waaler test: a titre of at least 1/40. (9) Positive acryl particle test: a ++ reaction at dilution 1/10 or higher. (10) Hypergammaglobulinemia: gammaglobulin concentrations above normal mean + 4 SD, the normal mean and SD were determined in the different laboratories where the paper electrophoresis tests were carried out by previous tests on healthy blood donors. Determination of IgG concentration in serum by the single radial diffusion technique has been described elsewhere (10).

(b) *Clinical syndromes:* (1) Lupus nephritis: the patients with SLE were defined as having lupus nephritis when both the urinary sediment and tests of renal function were abnormal. (2) Acute lupus skin lesions: the following conditions were included in this term: butterfly rash or eruption, nail bed changes, palmar erythema, photosensitivity and maculopapular lesion of lupus nature (cf Table V). (3) Chronic lupus skin lesions: both localized and generalized discoid lupus were regarded. (4) Marked arth-

the patients with SLE were defined as having marked arthritis when they had at least 5 of the American Rheumatism Association's criteria for rheumatoid arthritis (RA) (5).

A. Annual incidence and prevalence rates of SLE

For the calculation of incidence and prevalence rates, only patients living and diagnosed inside the area were considered, the rates being adjusted for increase of population (24). Two patients excluded from the SLE material were included when calculating the rates: one patient refused to cooperate for the family study, the other was a physician who was not informed about the diagnosis of the disease from which he was suffering. Two female patients in the SLE proband material resided outside the area and were not included when calculating the rates.

The annual rates of newly diagnosed definite SLE cases in the years 1959–1963 are given in Table I.

SLE patients diagnosed prior to and still living in 1959 were computed in the prevalence rate. Forty-six new definite SLE cases were diagnosed within the area in the period 1959–1963. The incidence per million varied from 4.1 in 1961 to 10.3 in 1962. The prevalence rate gradually increased from 19.6

Table I Incidence and prevalence rates of SLE in central eastern Norway

Year	N of new patients with definite SLE	Incidence	Prevalence
1959	8	5.6	19.6*
1960	7	4.9	22.2
1961	6	4.1	25.5
1962	15	10.3	31.5
1963	10	7.5	36.5

* No. of cases per million.

per million in 1959 to 36.5 per million in 1963. The Zinkham-Conley test (28) was employed by more hospitals after 1961 and the increased incidence was mainly due to the introduction of this LE cell test. The gradual increase of prevalence of SLE during the period was a consequence of the increased incidence but was also due to longer survival of the patients.

B. The course and duration of disease

The sex ratio was about 1:6.11 (18.9 per cent) of the 72 patients being males and 61 (84.7 per cent) females. The age at onset, the diagnosis, the death, and the sample study are presented for the two sexes in Table II.

Table II Age distribution of 72 patients with SLE

	No. of patients/Decades							
	0–9	10–19	20–29	30–39	40–49	50–59	60–69	>70
Females								
At onset	1	3	18	17	14	10	2	
At diagnosis		3	7	10	21	12	6	2
At death		1	4	6	7	1	6	
At sample study		1	3	7	17	10	5	1
Males								
At onset		2	3	1	2		3	
At diagnosis		1	3	1	2	1	3	
At death			1	2		1		
At sample study		1		1	2	3		

The age at onset refers to when the patients first exhibited manifestations which logically developed into a more definite clinical picture (4) and the age at diagnosis refers to the time when available data fulfilled the diagnostic criteria for definite SLE.

Females: 45 (70.8 per cent) of the 63 patients had their first symptoms in the third to the fifth decade and the main proportion (70.5 per cent) were diagnosed in the fourth to the sixth decade. 25 (40.9 per cent) died during the present study i.e., up to 1966.

Males: The age at onset and diagnosis was more widely distributed than in females. Four (36.4 per cent) of the 11 male patients died during the period of study.

Eleven (15.3 per cent) of the 72 patients experienced an acute onset of the disease the diagnosis being established within 6 months, in 21 cases the diagnosis was made within 18 months. Most patients experienced a gradual onset of the disease with an interval between onset and diagnosis of three years or more. Generally the prognosis was poorer in cases diagnosed within years. Fourteen (19.4 per cent) of the 72 patients died within 18 months of diagnosis.

The figures are shown in Table III.

C. Clinical manifestation

SLE is a chronic disseminated disease with tendency to exacerbations and remissions (8). Most organs of the body are affected. Generally gradual onset develops into an active

phase followed by remission. Relapses of varying intensity and remissions, usually occur prior to and after the diagnosis of SLE.

The frequency of various clinical manifestations seen in the 72 SLE patients is given in Table IV. The incidence at onset and at diagnosis is shown together with the cumulative incidence.

Acute renal insufficiency was present in 11 patients (15.3 per cent) at the time of diagnosis, and the cumulative percentage was 29. Generally chronic renal failure developed after repeated or severe acute attacks, uraemia occurred in 18 of the cases. Hematuria and proteinuria without renal insufficiency were found in higher percentages. Pulmonary lesions were observed in about 3/4 of the patients. Eleven (15.3 per cent) of the 72 SLE patients had thyroid disease: hyperthyroidism 4, colloid goiter 3, non-toxic adenoma 2, hypothyroidism 1 and subacute thyroiditis 1.

The frequency of various clinical manifestations usually attributed to vascular lesions (4) is seen in Table V.

Acute lupus skin lesions were present at onset in 15 (18.1 per cent) and chronic skin lesions in 4 (5.6 per cent) of the patients. Usually the skin lesions were of the same kind throughout the course in each patient. Except for more disseminated chronic forms, chronic discoid skin lesions ran their own course independent of variations in systemic involvement. This was observed in 6 probands, usually when the chronic discoid forms were localized to cheeks. Raynaud phenomena were an early and predominant feature in 11 of the 72 patients. Six of the 19 patients with Raynaud phenomena died of renal failure. Cytoid bodies were diagnosed in single case.

D. Hematological findings

A survey of hematological findings is presented in Table VI. Percentages are shown for the number of patients tested.

Anemia was present in 48 (66.7 per cent) of the patients, leucopenia in 20 (27.8 per cent) and thrombocytopenia in 18 (25 per cent).

Direct LE cell tests (all phenomena included) were positive in all but two patients who were tested in uremic phase. A typical related typical wire loops in both these patients.

TABLE III Duration of disease in 72 patients with SLE

Months	From onset to diagnosis		From diagnosis to death	
	Female	Male	Female	Male
0-6	9		4	
7-12	7	3	8	
13-36	13	1	3	
37-60	6	1	5	1
61-84	6	1	3	1
85-106	8	1	1	
107-132	3	1	1	
>133	9	1		

Table IV Frequency of various clinical manifestations in 72 patients with SLE.

	At onset	At diagnosis	Cumulative incidence
Fever > 38.5 °C	16 (22.2 ^{***})	50 (69.4)	61 (84.7)
Intermittent fever	15 (20.8)	39 (54.2)	47 (65.3)
Myocarditis	0	10 (13.9)	18 (25.0)
Pericarditis	0	19 (26.4)	30 (41.7)
Nephritis (acute)	1 (1.4)	11 (15.3)	21 (29.2)
Proteinuria	2 (2.8)	36 (50.0)	50 (69.4)
Hematuria	1 (1.4)	21 (29.2)	29 (40.2)
Casts	0	12 (16.7)	19 (26.4)
Ureaemia	0	5 (6.9)	18 (25.0)
Hepatosplenomegaly	2 (2.8)	7 (9.7)	20 (27.8)
Splenomegaly	1 (1.4)	8 (11.1)	12 (16.7)
Lymphadenopathy	4 (5.6)	19 (26.4)	30 (41.7)
Severe anorexia	11 (15.3)	17 (23.6)	27 (37.5)
Fatigability	2 (2.8)	38 (52.8)	49 (68.1)
Pulmonary lesions	3 (4.2)	28 (38.9)	56 (77.8)

N = number of patients with the symptom.

*** Percentage of patients with the symptom.

Cumulative incidence refers to the time of sample study or death.

Table V Frequency of clinical manifestations attributed to vascular lesions in 72 patients with SLE.

	At onset	At diagnosis	Cumulative incidence
Butterfly rash	2 (2.8)	6 (8.3)	11 (15.3)
Butterfly eruption	0	6 (8.3)	7 (9.7)
Nail bed changes	0	2 (2.8)	4 (5.6)
Palmar erythema	4 (5.6)	5 (6.9)	6 (8.3)
Photosensitivity	5 (6.9)	10 (13.9)	13 (18.1)
Maculopapular lesions	2 (2.8)	16 (22.2)	23 (31.9)
Discoid lupus - localized to cheeks	3 (4.2)	2 (2.8)	3 (4.2)
Discoid lupus - generalized	1 (1.4)	5 (6.9)	7 (9.7)
Alopecia	3 (4.2)	5 (6.9)	7 (9.7)
Purpura, ecchymosis	3 (4.2)	15 (20.8)	23 (31.9)
Urticaria	6 (8.3)	9 (12.5)	16 (22.2)
Raynaud phenomenon	8 (11.1)	18 (25.0)	19 (26.4)
Neuritis	0	7 (9.7)	11 (15.3)
Arteritis of central nervous system	1 (1.4)	3 (4.2)	6 (8.3)
Epilepsy	1 (1.4)	1 (1.4)	5 (6.9)
Cystoid bodies	0	0	1 (1.4)
Hemorrhages (ocular fundic lesions)	0	3 (4.2)	4 (5.6)

The figures are recorded as in Table IV

Table VI Frequency of hematological aberrations in 72 patients with SLE

	At onset	At diagnosis	Cumulative incidences
Anemia	15 (21.1)	37 (51.4)	48 (66.7)
Hemolytic anemia	0	6 (8.3)	7 (9.7)
Leucopenia	1 (1.4)	11 (15.3)	20 (27.8)
Thrombocytopenia	0	12 (16.7)	18 (25.0)
Numerous LE cells	1 (2.4)	51 (71.8)	60 (83.3)
Few LE cells	1 (2.4)	3 (4.2)	3 (4.2)
Few LE cells and ECM	0	3 (4.2)	2 (2.8)
ECM	0	2 (8)	3 (6.9)
Wheeler test positive	0	9 (13.6)	14 (19.6)
False loss serology	5 (6.9)	14 (19.6)	14 (19.6)
Hypergammaglobulinemia	2 (4.7)	34 (47.9)	36 (50.7)
Positive antinuclear test	0	7 (13.5)	10 (13.9)
Cold agglutinins	0	1 (1.9)	3 (6.9)

The figures are recorded as in Table IV

tients. Numerous LE cells were seen in 60 of the cases. The direct LE cell tests were only weakly positive in 10 of the patients but 5 of these had titres of 1/4 or higher in the indirect LE factor test. The indirect LE factor test was positive in 59 (76.5 per cent) of the 51 patients with SLE personally examined by the author. However the disease was not necessarily active during blood sampling. This test is described in detail in a separate paper (11). One patient with only extracellular homogeneous material (ECM) had a titre of LE factor of 1/64 by the indirect test and above 1/1000 of antinuclear factors detected by immunofluorescent

The gamma-globulin examined by paper electrophoresis was increased in 36 of the patients (mean value ± 4 SD). It decreased during remission.

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The following variables were significantly increased in this group: lymphadenopathy ($p = 0.0324$), skin hemorrhages ($p = 0.0398$), butterfly rash and eruption ($p = 0.008$), intermittent fever at diagnosis ($p = 0.04$), onset of SLE before menopause ($p = 0.017$) and mortality ($p < 0.001$).

SLE patients without lupus nephritis had significantly increased: myocarditis at diagnosis ($p = 0.04$), bronchitis ($p = 0.0242$), roentgenographic evidence of erosive arthritis at diagnosis ($p = 0.02$) and IgG concentrations ≥ 1740 mg/100 ml ($p = 0.04$).

Hypergammaglobulinemia. Thirty-six (50.7 per cent) of the patients examined had hypergammaglobulinemia.

This group of patients was characterized by severe symptoms of the disease with high fever, pleurisy, pulmonary involvement, and rheumatoid symptoms with and without skin lesions. Generally distributed. Acute onset. The frequency of hypergammaglobulinemia was 36 (50.7 per cent) in the acute phase and 34 (47.9 per cent) in the chronic phase.

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E. S. J. *et al.* SLE syndrome. The disease was classified during various examinations with a specified Lupus developed forms).

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Table VII. Frequency of rheumatic diseases in 72 patients with SLE.

	At onset	At diagnosis	Cumulative incidence
2 of ARA criteria for RA	17 (23.7)	11 (25.0)	12 (16.5)
3-4 of ARA criteria for RA	11 (26.4)	21 (29.2)	27 (37.5)
5-6 of ARA criteria for RA	6 (8.3)	16 (22.2)	17 (23.6)
7-8 of ARA criteria for RA	0	5 (6.9)	7 (9.7)
Röntgenological changes in joints	3 (4.6)	13 (19.7)	18 (25.0)
Keratconjunctivitis sicca	1 (1.4)	5 (6.9)	6 (8.3)
Effusions in small joints (toes and fingers)	24 (33.3)	24 (33.3)	45 (62.5)
Effusions in large joints (wrists, elbows, shoulders, knees and ankles)	28 (38.9)	29 (40.3)	50 (69.4)
Diffuse muscular pains	13 (18.1)	29 (40.3)	33 (45.8)
Ankylosing spondylitis	0	0	1 (1.4)
Rheumatic fever	5 (6.9)	0	12 (16.7)
Goiter	1 (1.4)	2 (2.8)	3 (4.6)

The figures are recorded as in Table IV

Patients without cut skin lesions of lupus nature had increased frequency of effusions in small ($p = 0.02$) and large ($p = 0.009$) joints at diagnosis.

Marked arthritis. One-third of the SLE cases had at least 5 of ARA criteria for RA besides the lupus features. The frequency of rheumatic diseases in the 72 SLE patients is given in Table VII.

Initially roentgenological evidence of erosive arthritis was found in 3 (4.6 per cent) of the 72 patients, but these patients later developed clinical pictures consistent with definite SLE. However all ran a favourable course except for their arthropathy. About one-quarter of the SLE cases had swellings in small joints (toes and fingers) both at onset and at diagnosis. The cumulative percentage of small joint effusions was 62.5. Effusions in joints were more frequent prior to than after SLE diagnosis, largely due to corticosteroid therapy.

SLE patients who developed marked arthritis had significant increase of the following variables: effusions in small joints prior to ($p = 0.0006$) and after SLE diagnosis ($p = 0.0396$); effusions in large joints (wrists, elbows, shoulders, knees and ankles) prior to ($p = 0.0016$) and after SLE diagnosis ($p = 0.0015$); Raynaud phenomena at diagnosis

($p = 0.005$); positive acryl particle test ($p = 0.015$) and positive Waaler test ($p < 0.001$).

SLE patients without marked arthritis had significant increase in the variables: intermittent fever at onset ($p = 0.0312$) and acute nephritis prior to diagnosis ($p = 0.018$).

Causes of death and autopsy findings

Of the 72 patients, 29 (40.3 per cent) died during the present study mainly of renal insufficiency. One female patient died in the active stage of SLE, but autopsy did not reveal renal damage.

Infectious diseases (pneumonia, septicemia and meningitis) were regarded as the cause of death in 9 patients. Four died of cardiac failure.

Autopsy was performed in 18 patients with the following findings: wire-loops (88.9 per cent); onion skin lesions (77.8 per cent); fibrinoid necrosis (44.4 per cent); vasculitis (44.4 per cent); myocarditis (27.8 per cent); hematoxylin-bodies (22.2 per cent); Libman-Sacks endocarditis (16.7 per cent) and amyloidosis (5.6 per cent).

DISCUSSION

There is no general agreement on what SLE is, and the diagnostic criteria are arbitrary (23). In the present study no definite criterion was employed, and all available data relevant for diagnosis of SLE according to data given in the literature were considered. Syndromes which were not regarded as typical SLE were excluded. The 72 patients given the diagnosis SLE were compared with and classified according to diagnostic criteria given in 6 papers (6, 9, 13, 20, 25, 27): 72 (100 per cent) would have been accepted as definite SLE patients by the criteria given in 3 out of these 6 references papers, 69 (95.8 per cent) by the criteria in 5 of the papers, and 63 (87.5 per cent) by the criteria given in all six papers. Six of the latter 9 SLE cases would have been excluded by the criteria used in one paper (13) mainly due to a different view concerning the significance of necropsy findings and the LE cell phenomenon.

The method of selecting the SLE cases was based on the assumption that the difficulty of diagnosis and the clinical severity of SLE would lead to hospitalisation at some time (23). This was apparently not always the case. Thus, one male patient who had not been hospitalised was diagnosed as SLE by the author during the family studies. This will contribute to the fact that the rates reported are in reality too low.

When the condition of the SLE patients deteriorates, the diagnosis of SLE depends upon the diagnostic criteria used and on how often the disease is considered likely. Generally the diagnosis of SLE was considered when the patients exhibited systemic manifestations, but the facilities for hematological and histological investigations varied from hospital to hospital. When the Zinkham-Conley test (28) — which is a sensitive and reliable LE cell test (8) — was employed by more hospitals the incidence of new SLE cases increased markedly.

The annual incidence and prevalence rates of definite SLE calculated for central eastern Norway in 1959–1965 were lower than the rates previously reported from other countries (13, 18, 22, 25).

SLE is generally regarded as a chronic disseminated disease with tendency to exacerbations and remissions. In the present study symptoms and signs at different phases of the

disease were computed separately but a cumulative incidence was also calculated.

By classifying SLE cases according to the clinical syndromes they exhibit during active disease a better understanding of the factors involved in different phases of the disease may be obtained. For example in SLE patients who developed lupus nephritis had increased frequency of skin hemorrhages, butterfly rash or eruption the onset of SLE was before menopause fever was marked at the time of diagnosis of SLE and the mortality was high, but they had decreased frequency of bronchitis and myocarditis and roentgenological evidence of erosive arthritis at diagnosis of SLE and the IgG concentrations in serum were lower. In SLE patients with hypergammaglobulinemia more often had a severe onset with a clinical picture of high fever pleurisy myocarditis and pulmonary infiltrations. In the SLE patients who developed marked arthritis during the disease differed in several respects from the patients who did not become arthritic.

A search for concordance or discordance of the variables at different phases of the disease appears to be valuable. Thus, in many cases, some of the lupus features, including organ involvements and laboratory findings, seemed to run their own course independent of each other. Except for more disseminated chronic forms, chronic discoid skin lesions ran their own course independent of variations in systemic involvement. The disease of patients with less specific criteria, like discoid skin lesions, marked arthritis and myocarditis, or chronic biologic false-positive test for syphilis, hemolytic anemia and purpura hypergammaglobulinemia, often ran a favourable rather chronic course.

The symptomatology in the presently described patients with SLE is consistent with previous reports (2 for references, see 4, 12). However the figures given in the literature vary. The natural history of SLE in Norway also confirms previous reports (14, 15) but no sex difference in the prognosis was observed in the present study.

The present material serves as proband material for family studies in SLE. The main purpose of this work was to investigate the clinical and serological aberrations in families of SLE patients. The description of SLE given

here is used as basis for the discussion of the abnormal findings in the families of the patients.

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II Development of an indirect LE factor test

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ABSTRACT

An indirect LE factor technique, including a simple method for separation of leucocytes, is described. In the test a standardized amount of serum reacts with a constant amount of damaged nuclei serving as antigen, and phagocytosis is secured by addition of a constant amount of freshly-prepared leucocytes. It is possible to titrate LE sera. The titration of LE sera gives a regular succession of different phenomena associated with LE cell. This gradual change in the morphology has been described. Granulocyte- and lymphocyte-specific LE factors are demonstrated.

The LE cell phenomenon was originally described in bone marrow (11) later in venous blood (12-25) and by addition of LE plasma to bone marrow (13). Investigations into this phenomenon, with elucidation of its mechanism have contributed greatly to our understanding of the immunological aberrations in systemic lupus erythematosus (SLE).

Three materials interact in the development of this phenomenon: the LE factor occurring in the patient's plasma, interacts with constituents in damaged nuclei. This leads to morphological changes of the nuclear substance with formation of LE bodies. Finally these are phagocytized by living granulocytes with formation of LE cells.

The LE cell phenomenon may be demonstrated by direct techniques when serum and leucocytes originate from the patient (for references, see 8). In indirect techniques, serum from the patient interacts with leucocytes from another source.

The initial phase of the LE phenomenon, i.e. the reaction between the LE factor and nuclear substance does not need complement (1-17). Complement is essential however for optimal phagocytosis, and formation of true LE cells.

The LE cell phenomenon has varied and rather complex morphology and the components have been described by different names.

Detailed information is available in reference 5. The LE factor behaves as a typical antibody directed against antigens present in nucleoprotein, requiring both DNA and histone for complete activity (18).

The purpose of the present investigation was to develop techniques for evaluation of the LE cell phenomenon in material of patients with systemic lupus erythematosus and their relatives. This required the use of sera with a possibility of determining the titre of the activity and parallel tests with known and serial dilutions of positive control sera. A constant source of antigen was also required. The present paper describes an indirect technique for demonstration of the LE factor which fulfills these criteria.

MATERIALS AND GENERAL TECHNIQUES

Sera. Sera were obtained from patients with systemic lupus erythematosus and their relatives, as described elsewhere (19-20). The sera were stored at -20°C in small aliquots and thawed once immediately prior to testing.

Separation of leucocytes: The technique and theory for separation of leucocytes has previously been described in detail (3). The separation of isolated granulocytes and isolated lymphocytes has also been described elsewhere (4).

Isopaque (natrii-N-methyl-3,5-diacetamid-2,4,6-trioxybenzoas) 75 per cent was provided by Nyegeard and Co., Oslo Norway and dextran 500 or dextran 250 were obtained from Pharmacia, Uppsala, Sweden. A stock solution containing 10 volumes Ispaque 33.9 per cent and 50 volumes dextran 250 was prepared, this solution has a density of 1.08 g per ml and is stored at 4 C. Two or three ml of the Ispaque-dextran stock solution were added to tubes of 100 x 15 mm and a similar amount of newly-drawn blood containing 10 IU heparin per ml was layered over the stock solution. The erythrocytes formed clumps which sedimented to the bottom of the tube, leaving a plasma layer containing 6-8000 leucocytes per mm³.

The separated leucocytes were used in the test both as a source of antigen and as phagocytes as follows:

Antigen. 1) Preparation Leucocytes were damaged by freezing and thawing. 2) Standardization: Heparinized blood from healthy group O donors was used for preparation of leucocyte-rich plasma which was frozen in small aliquots after counting the leucocytes. They were thawed immediately before use and diluted by heparinized pooled normal serum containing 20 IU heparin per ml. The amount of antigen in the test mixture was kept constant by varying the amount of normal serum. This is required so as to have constant conditions in the test, as described below. The antigen should be used immediately after thawing. 3) Storage: Storage of antigen in the frozen state should not be for more than 3 weeks (vide infra).

Phagocytes. Leucocytes in the plasma layer were also used as living cells for phagocytosis and thus formation of LE cells. The cells were used immediately after separation, but survived storage overnight at 4 C. The number of phagocytosing cells was standardized to obtain constant conditions during the test.

Performance of the test. Half millilitre test serum 0.2 ml antigen (containing 5×10^6 nuclei) and 0.3 ml fresh leucocyte-rich plasma (containing $\approx 10^6$ leucocytes) were added to a small test tube (80 mm x 10 mm). The tube was stoppered, shaken to ensure complete mixing of the contents and kept at 37 C for 90 minutes. The tubes were gently shaken 2-3 times during this incubation period. After incubation, the tubes were centrifuged for 4

minutes at 600 g at 20 C. The supernatant was removed and the thin cellular layer on the bottom of the tube harvested by a thin, dry Pasteur pipette. Two drops were placed on glass slides and smeared between two slides to obtain a concentrated and single layer of intact cells (10). Complete removal of the supernatant fluid prior to harvesting the cells is important. Careful preparation of the smear is also an important requirement for optimal evaluation of the LE cell phenomenon.

Positive and negative controls were always run in parallel, negative controls being selected normal sera and positive controls sets of dilutions of known positive sera.

Staining: The slides were first incubated with methanol for 15-30 minutes, and then with May-Grunwald stain (A.G. Merck, Darmstadt, Germany 1 volume and 1 volume 0.2 M phosphate buffer of pH 6.8) for 15 minutes. Final incubation was with Giemsa stain (A.G. Merck, Darmstadt, Germany 1 volume plus 9 volumes of the phosphate buffer) for 5 minutes. The slides were then washed twice in the phosphate buffer.

RESULTS

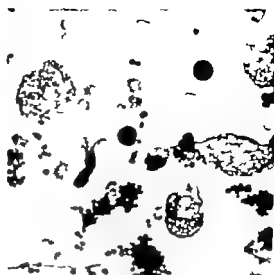
In the test, a standardized amount of serum reacted with a constant amount of damaged nuclei serving as antigen, and phagocytosis was secured by addition of a constant amount of freshly-prepared living leucocytes. It is established that the size form and staining of the LE body is dependent on the amount of LE factor reacting with the nuclei (9). With the present technique, the nuclei were damaged by prior freezing and thawing. This leads to a constant degree of damage to the nuclei, and a similar amount of LE factor was bound to each damaged nucleus. As a result, the morphology was virtually constant throughout each preparation, which made the microscopic reading rapid and simple.

Morphology of the LE cell phenomenon in the described test

When LE factor was absent, the nuclei remained unchanged and were seen as nuclear material forming long strands between the intact



Fig. 1 Negative LE phenomenon. A. The nuclei are unchanged and the nuclear material forms long strands



between the intact living leucocytes. B. The nuclei are swollen, but the chromatin intact (ca. $\times 420$).

living leucocytes. This is illustrated in Fig. 1A. Sometimes, dense small, irregularly-shaped nuclei were seen, and, more rarely swollen nuclei with intact chromatin (Fig. 1B).

Large amounts of LE factor usually resulted in formation of numerous LE cells throughout the preparation. The appearance of the preparation is an indication of the amount of LE factor present in the serum but better information is obtained through titration of the serum and determination of a titre which corresponds to the highest dilution of serum giving distinctly positive phenomenon.

The gradual change in the morphology of the LE cell phenomenon is illustrated in Fig. 2 by the findings using different dilutions of strongly positive LE serum R.M., obtained from female patient with systemic lupus erythematosus.

Fig. 2A illustrates the morphology when the serum is diluted 1:4. Serum R.M. shows prozone phenomenon when used undiluted, as only LE bodies are formed. There are clusters of classical LE cells with multiple inclusions of various size; rosettes and LE bodies are rare. The inclusion bodies are seen in granulocytes. When round homogeneous LE bodies are present, they are small.

When the serum is diluted 1:100 a shift

from LE cells to rosettes is seen (Fig. 2B). The rosettes consist of leucocytes around LE bodies which are usually larger and less homogeneous than free LE bodies. Some of the inclusion bodies of LE cells are also less homogeneous.

Fig. 2C shows forms of rosettes obtained when the serum is diluted 1:400. The LE bodies within the rosettes are usually disintegrated; these true rosettes are sometimes difficult to discriminate from false forms formed by leucocytes around other materials, usually platelets.

Fig. 2D shows LE bodies which are irregularly-shaped and less homogeneous. Some LE bodies are attached to leucocytes and disintegrated at the site of attachment. The conditions for complete rosette formation are not present, as the serum is diluted 1:800.

When serum R.M. is further diluted, the shape of the LE bodies becomes even more irregular and less homogeneous. The titre is 1:3200 and corresponds to the highest dilution giving a distinct nuclear change. Finally unchanged nuclear material is seen between the intact living leucocytes. Parallel tests with known negative sera sustain these negative findings.

The different components of the LE cell phenomenon vary in morphology. Fig. 3 shows LE cells with a coarser structure of the inclu-

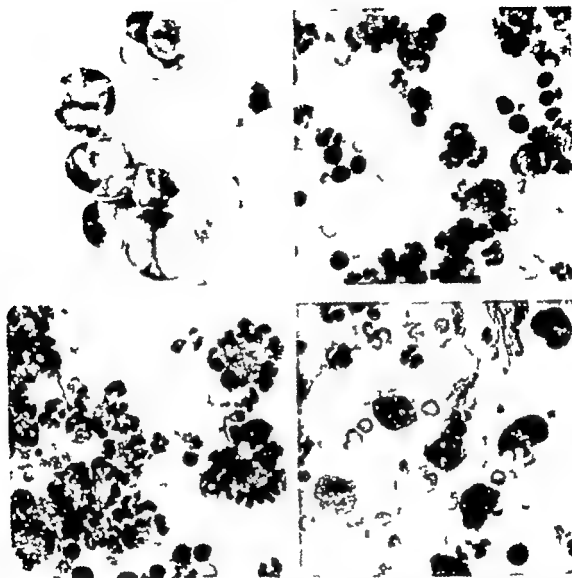


Fig. 2. Positive LE phenomena. The various morphological phenomena appear with increasing dilution of a strong LE serum, obtained from female SLE patient R.H.I. A. *N. nervos*-associated LE cells. Note the clusters of multiple inclusions of various size. Serum 1:4 (ca. \times 900). B. LE cells and rosettes. Note the shift from LE cells only to LE cells and rosettes. Rosettes consist of leucocytes around LE bodies. Serum 1:300 (ca. \times 420). C. *R. setosus*. LE bodies within the rosettes are dis-

tinguished. True rosettes are sometimes difficult to discriminate from clumps formed by leucocytes around other materials, usually platelets. Serum 1:400 (ca. \times 420). D. LE bodies. Note that the LE bodies are larger, more irregularly shaped and less homogeneous than the LE bodies shown in Fig. 4A. Some LE bodies are attached to leucocytes, but complete rosette formation is absent. Serum 1:800 (ca. \times 420).

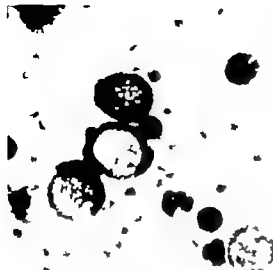


Fig. 3. Atypical LE cells. Note the coarse structure of the inclusion bodies. This strong LE serum M.G. was obtained from a female SLE patient with uremia, the LE bodies exhibit the same appearance (ca. 900).

sion bodies than usually seen. This was obtained with serum M.G. diluted 1:2 from female SLE patient with uremia, the LE bodies exhibit the same appearance even when the serum is further diluted. The titre is 1:64. The patient had similar titre early in active disease but the morphology of the LE cell phenomenon was at that time like that illustrated in Fig. 2A-D.

Fig. 4A shows classical round homogeneous LE bodies; sometimes the LE bodies melt together and form large irregular shapes. LE sera giving such large bodies rarely exhibit LE cells.

Fig. 4B shows LE bodies of lacy, reticular appearance which are usually seen when the LE phenomenon is weakly positive. Sometimes the lacy round LE bodies form larger bodies.

The influence of varying the conditions for the test.

Selection of the amount of serum damaged nuclei and other conditions for the test were based upon systematic experiments, the conditions for the test being varied one at a time. Some of this information will now be described.

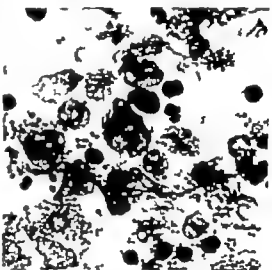
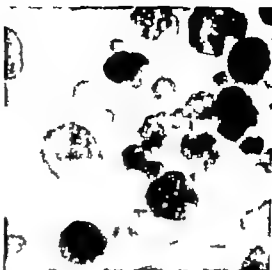


Fig. 4. Types of LE bodies. A. Classical round homogeneous LE bodies. Note the shape and size of the LE bodies. This type is observed when larger amounts of LE factor are present (ca. 900). B. Lacy LE bodies. These are usually found when small amounts of LE factor are present. The LE bodies are different from those shown in Fig. 2D, which illustrate distinctly positive LE phenomenon, and from the swollen nuclei seen in Fig. 2B, which illustrate negative LE phenomenon (ca. 420).

Table I. Titres of various morphological LE phenomena when different amounts of nuclei (antigen) were incubated with a constant amount of serum.

Leucocyte nuclei per ml LE serum	Reciprocal titre		
	LE bodies	Rosettes	LE cells
2.8×10^6	400	200	200
1.4×10^6	1600	800	400
0.7×10^6	3200	1600	800
0.2×10^6	3200	—	3200

Table I shows the findings when one serum (R.M.) was incubated with varying amounts of damaged leucocytes. It is evident that the titre decreased for formation of LE bodies, rosettes and complete LE cells when the amount of nuclei was increased relative to the amount of serum. The amount of damaged nuclei used in the test was selected as the amount providing optimal sensitivity of the test. Under special conditions where increased sensitivity is desirable, the amount of serum relative to damaged nuclei can be increased over that specified.

Table II illustrates that the properties of the antigen changed during storage at 22 C. This effect was not due to development of anticomplementary activity since the other morphological features of the LE phenomenon were similarly affected. Antigen stored for less than 3 weeks was therefore used in the test.

The morphological appearance also depended on the number of living leucocytes relative to the amount of damaged nuclei. Table III shows the results of some experiments where the amount of serum was kept constant, while the numbers of living leucocytes and nuclei were varied. When the amount of damaged

Table II. The effect of storage of antigen at 22 C on the LE cell phenomenon induced by a strong LE serum R.M.

Storage of antigen in weeks	Reciprocal titre of LE cells
0	800
2	800
4	200
8	200
10	50
14	25
16	5
20	5
24	<1

nuclei was decreased relative to the numbers of leucocytes, it led to formation of more complete LE cells relative to LE bodies. When the numbers of living leucocytes were also lowered, a decreasing amount of damaged nuclei led to formation of even more complete LE cells in relation to LE bodies.

One aspect of the importance of selection of antigen donor is shown in Table IV. It is evident that the formation of LE bodies, rosettes and complete LE cells varied in different LE sera when the amount of nuclei was decreased relative to the amount of serum.

Isolated granulocytes and isolated lymphocytes were used for preparation of antigen, and then tested with a series of known positive sera. Table V shows that the titres of 5 sera varied considerably when tested with granulocytes and lymphocytes respectively. Serum E.L. showed a higher titre against granulocytes than lympho-

Table III. The ratio of LE cells/LE bodies when nuclei and leucocytes are gradually decreased

Leucocyte nuclei per ml LE serum	Living leucocytes per ml LE serum			
	5×10^6	2.5×10^6	1.25×10^6	0.6×10^6
3.6×10^6	<0.01	0.3	0.5	1.0
1.8×10^6	0.01	0.5	2.0	3.0
0.9×10^6	0.02	0.5	2.0	>10
0.45×10^6	0.04	0.5	2.0	>10
0.22×10^6	0.10	0.5	—	>4

Table IV Various morphological LE phenomena in strong LE sera when different amounts of nuclei (antigen) were incubated with constant amount of undiluted serum

Leucocyte nuclei per ml LE serum	SLE patient		
	G. H.	A. L.	A. J.
2.8×10^6	LE bodies	LE bodies	LE bodies
1.4×10^6	LE bodies	Rosettes	LE bodies
0.7×10^6	Rosettes	LE cells	LE bodies
0.2×10^6	LE cells	LE cells	LE bodies

Table V The effect of using isolated granulocytes or isolated lymphocytes in the indirect LE factor test.

LE serum from	Reciprocal of titre with Nuclei of	
	Granulocytes	Lymphocytes
E. L.	4	<1
S. K.	<1	4
N. O.	8	8
K. H.	4	16
M. R.	16	8

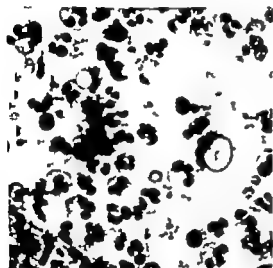


Fig. 5. Granulocyte-specific LE factor. A. Isolated granulocytes are used as antigen and tested with serum E.L. Note that LE cells are formed. B. Isolated lymphocytes are tested with serum E.L., but the nuclei are unchanged and are seen as strands (ca. 420).

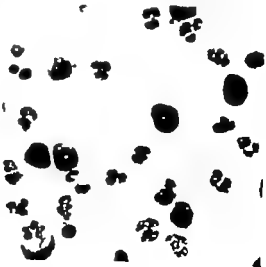


Fig. 6. Lymphocyte-specific LE factor. Isolated lymphocytes are used as antigen and tested with serum S.K. Note the LE bodies. When isolated granulocyte nuclei are tested with serum S.K., the nuclei are seen as in Fig. 4B (ca. 420).

cytes, whereas sera S.K. and K.H. showed higher titres against lymphocytes than against granulocytes.

Fig. 5A shows LE cells formed when isolated granulocytes were used as antigen and tested with serum E.L. When a similar amount of isolated lymphocytes from the same donor was tested with serum E.L., the nuclei remained unchanged, as shown in Fig. 5B. Serum E.L. also showed a positive antinuclear factor reaction only when granulocytes were used as antigen in the immunofluorescent technique. The granulocyte-specific ANFs of serum E.L. were kindly demonstrated by Dr. Elling, Auto-Immune Laboratory, Statens Seruminstitut, Copenhagen, Denmark.

Fig. 6 shows LE bodies when lymphocytes were used as antigen and tested with serum S.K. The nuclei of granulocytes were unaltered when tested with serum S.K.

DISCUSSION

The LE factor was originally defined as the factor that induced the morphologically typical LE cell phenomenon (14). Adequate criteria have been described for true LE cells (21) but other related morphological phenomena may be present alone or together with typical LE cells. The LE cell tests often provide conditions insufficient for optimal phagocytosis (6) and the significance of the other morphological patterns has not been established.

A quantitative assessment of the LE factor has previously been regarded as very difficult, particularly since the morphological phenomena may differ in various fields of the preparations (16). In the presently described experiments the leucocytes were damaged by freezing and thawing. This procedure was originally introduced by German & Heller (8) and used by Lachman in his two-stage indirect LE cell test (16). In the present technique there is a constant degree of damage of the nuclei and a similar amount of LE factor is bound to each damaged nucleus. The morphology is therefore constant throughout each preparation and it is possible to determine the titre of LE factor activity by the use of a standard serum.

antigen. The assessment of the LE factor in this indirect test is largely based on the effect of the first step of the LE phenomenon, namely the interaction between damaged nuclei and the LE factor. The same principle is applied in the loose body test (22). When the other conditions are kept constant, the various morphological phenomena appear regularly with increasing dilution of strong LE sera (9). Strong SLE sera sometimes show prozone phenomena, as only LE bodies are formed, but undiluted strong LE sera usually exhibit numerous inclusion bodies. The best conditions for phagocytosis were achieved when the tubes were incubated at 37°C for 90 minutes.

Antinuclear factors react with different nuclear antigens (2, 18, 24). Granulocyte and lymphocyte-specific ANFs have been described (7). This is probably a parallel to the present demonstration of granulocyte-specific and lymphocyte-specific LE factors. Cell specific LE factors are noted when the standard antigen which consists mainly of granulocytes is employed, but have to be sustained in additional tests with isolated granulocytes and isolated lymphocytes. Most frequently granulocyte specific LE factors are found.

The complex morphology of the LE phenomenon probably expresses qualitative and quantitative differences in the reaction between several LE factors and various antigenic structures in nuclei, and not interaction of an IgG antibody of limited specificity with nucleoprotein.

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III Presence of LE factor in relatives and spouses

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ABSTRACT

LE factor was determined in sera of 928 family members of 72 patients with SLE by an indirect LE factor test. The prevalence of LE factor was significantly increased in relatives compared with their spouses, female first degree relatives presenting the highest frequencies. The presence of LE factor in relatives was correlated to presence of chronic disease, but LE factor also occurred in apparently healthy relatives. The frequency of LE factor was independent of size of sibships and families and residence of the relatives.

The LE cell phenomenon is due to antinuclear antibodies reacting with nuclei of damaged cells (for references, see 18). This initially leads to morphological changes of the nuclei with formation of LE bodies. Subsequently the LE bodies are engulfed by living white blood cells to form LE cells. LE bodies, rosettes, and LE cells occur in LE cell preparations, but the significance of the various morphological elements is still disputed. LE cells are found in systemic lupus erythematosus (SLE) and related disorders (16).

Several to-antibodies, including antinuclear factors (ANF) occur with increased frequency in families of patients with SLE (for references, see 8). A test of LE factor has rarely been included in such studies (25-33, 34) mainly for technical reasons. As part of an extensive study of clinical and serological aberrations in relatives of patients with SLE the results of an indirect LE factor test are presented in this paper. A marked familial aggregation of LE factor was found, and LE factor was demonstrated in healthy individuals.

MATERIALS AND METHODS

SLE probands. The criteria for selection of the 72 probands with SLE have been published elsewhere (19). The probands represented all individuals considered to be definite SLE cases

in a defined area from central eastern Norway during the years 1959-1963.

The family material. Initially the SLE patients and their closest available family members (parents, spouse or children) were asked if they would participate in the present study and inform and persuade the other family members. Subsequently visits to the homes were arranged in cooperation with the initially contacted persons who accompanied the author on the visits. All available family members, irrespective of residence in Norway or adjacent parts of Sweden, completed questionnaire were clinically examined, and while in the fasting state provided 80 ml blood sample. First degree relatives (children, siblings and parents) second degree relatives (siblings of parents) and their spouses were primarily included in the investigation. The spouses of the SLE probands served as first degree controls together with the spouses of the siblings. Other relatives, mainly grandparents and grandchildren, were also included, when available. The pedigrees were submitted to and confirmed by the other family members, and additional data collected from their physicians files and hospital records. The data from the questionnaires, physicians and hospital records, clinical and serological examinations (about 700,000 in all) were punched on data cards for electronic data processing.

Table I Survey of relatives of the 72 SLE probands and their spouses.

Group	Sex	Tested and clinically examined		Refused to cooperate		Completion rates (%)		Not available but data present		Unknown,* data not present	
		Relatives	Spouses	Relatives	Spouses	Relatives	Spouses	Relatives	Spouses	Relatives	Spouses
First degree	female	191	78	3	15	98.5	83.9	44		40	23
	male	178	109	9	11	93.2	90.8	41		53	38
	both	369	187	12	26	96.8	87.8	85		93	61
Second degree	female	105	56	9	12	92.1	82.4	29	1	166	161
	male	97	53	15	5	86.6	91.4	38		172	175
	both	202	109	24	17	89.3	86.5	67	1	338	336
First and second degree	female	296	134	12	27	96.1	83.2	73	1	206	184
	male	275	162	24	16	91.9	91.0	79		225	203
Other	both	44	17					3			
Total	both	615	313	36	43	94.5	87.9	155	1	431	387

* Abroad or deceased

Most of the relatives had died shortly after birth, and the spouses were usually married to deceased relatives.

A survey of groups of relatives (of the 72 probands with SLE) and their spouses is given in Table I.

Approximately 63 per cent of first and second degree relatives were included in the present LE factor study but available information was present from an additional 165 relatives either abroad or deceased. Thus, data were present from 83 per cent of first degree relatives. Most of the relatives with no available information had died shortly after birth. The completion rates in the groups of relatives varied from 86.6 per cent to 98.5 per cent. The spouses of the SLE probands are included in Table I. The completion rates in groups of spouses varied from 82.4 to 91.4 per cent. These completion rates are in agreement with the claims for family studies (23). Unknown spouses were usually married to deceased relatives. The age and sex distribution of the relatives and spouses, all tested and clinically examined, is shown in tables II and III.

The ratio between relatives and spouse was

2:1

Statistical methods. The data were processed in a CDC 3300 computer at the University of Oslo, Norway. The age distribution usually differed in the various populations. ANF positivity is regarded as being age-dependent (2, 5, 32). χ^2 calculations were therefore performed separately for each age group and the total group (obtained by summing up the individuals in the various age groups) but significant differences (p-values below 0.05) between total groups were usually not taken into account. When the task was to combine evidence for the three age groups (sets of 2×2 tables) Mantel-Haenszel's method (M-II) was employed (28). A continuity correction is used (9, 28).

All p-values of significant level (< 0.05) are given in the tables. However when the p-values in specified groups seem to contribute to significant differences when groups are summarized, non-significant p-values are also indicated. In some instances, non-significant p-values are also given.

Indirect LE factor test. The technique has been described in detail elsewhere (18). The

Table II. Age and sex distribution of groups of relatives for the 72 SLE probands.

Group	Sex	Age distribution (yrs)			T total
		<40	40-59	≥60	
Offspring	female	34 (94.4%)	2 (5.6)	0	36 (100.0)
	male	39 (88.6)	5 (11.4)	0	44 (100.0)
Siblings	female	34 (28.3)	62 (51.7)	24 (20.0)	120 (100.0)
	male	22 (21.5)	60 (58.9)	20 (19.6)	102 (100.0)
Parents	female	0	8 (22.9)	27 (77.1)	35 (100.0)
	male	0	7 (21.9)	25 (78.1)	32 (100.0)
Total first degree	female	111 (35.6)	72 (37.7)	51 (26.7)	191 (100.0)
	male	61 (34.2)	72 (40.5)	45 (25.2)	178 (100.0)
	both	129 (34.9)	144 (39.0)	96 (26.0)	369 (100.0)
Sib of mother	female	1 (1.5)	23 (33.8)	44 (64.7)	68 (100.0)
	male	3 (5.5)	23 (41.8)	29 (52.7)	55 (100.0)
Sib of father	female	0	11 (29.7)	26 (70.3)	37 (100.0)
	male	1 (2.4)	15 (35.7)	26 (61.9)	42 (100.0)
Total second degree	female	1 (1.0)	34 (32.4)	70 (66.6)	105 (100.0)
	male	4 (4.1)	38 (39.2)	55 (56.7)	97 (100.0)
	both	5 (2.5)	72 (35.6)	125 (61.9)	202 (100.0)
First and second degree	female	69 (23.3)	106 (35.8)	121 (40.9)	296 (100.0)
	male	65 (23.7)	110 (40.0)	100 (36.3)	275 (100.0)
Other	both	24 (54.5)	9 (20.5)	11 (25.0)	44 (100.0)
Total	both	158 (25.7)	225 (36.6)	232 (37.7)	615 (100.0)

No. of individuals in the specified age group

Percentage individuals in the specified age group

Sera were coded, stored in small aliquots at 22°C, thawed once before use, recorded by technical assistant, and the tests read by double blind technique. A standardized antigen from single healthy blood donor was employed; the antigen was stored for less than three weeks before use (18). Positive sera were tested in two fold serial dilutions, and weakly positive sera were retested. All tests were performed in duplicate. Positive and negative

controls were included every day. The criteria for positive tests and determination of titres have been described in detail elsewhere (18). The last dilution which induced morphological changes of the nuclei making them distinctly different from the negative (normal) control determined the titre. Sera with titre of 1/1 and higher in the indirect LE factor test were considered as positive.

Table III Age and sex distribution of controls (spouses of relatives of the 72 SLE probands).

Group	Sex	Age distribution (yrs)			Total
		<40	40 - 59	≥60	
Offspring	female	7 (77.8)	2 (22.2)	0	9 (100.0)
	male	4 (40.0)	6 (60.0)	0	10 (100.0)
Siblings	female	22 (31.9)	39 (56.5)	8 (11.6)	69 (100.0)
	male	19 (19.2)	81 (65.7)	11 (15.2)	99 (100.0)
Total first degree	female	29 (37.2)	41 (52.6)	8 (10.2)	78 (100.0)
	male	23 (21.1)	71 (65.1)	15 (13.7)	109 (100.0)
	both	52 (27.8)	112 (59.9)	23 (12.3)	187 (100.0)
Sub. of mother	female	2 (6.7)	16 (53.3)	12 (40.0)	30 (100.0)
	male	0	10 (25.0)	30 (75.0)	40 (100.0)
Sub. of father	female	2 (7.7)	18 (61.5)	8 (30.8)	28 (100.0)
	male	0	5 (38.5)	8 (61.5)	13 (100.0)
Total second degree	female	4 (7.1)	32 (57.1)	20 (35.8)	56 (100.0)
	male	0	15 (28.3)	38 (71.7)	53 (100.0)
	both	4 (3.7)	47 (43.1)	58 (53.2)	109 (100.0)
First and second degree	female	33 (24.6)	73 (54.5)	28 (20.9)	134 (100.0)
	male	23 (14.2)	86 (53.1)	53 (32.7)	162 (100.0)
Other	both	8 (47.1)	5 (29.4)	4 (23.5)	17 (100.0)
Total	both	64 (20.4)	164 (52.4)	85 (27.2)	313 (100.0)

The figures are recorded as in Table II

RESULTS

A. The prevalence of LE factor in relatives and spouses.

The prevalence of LE factor in the relatives is shown in Table IV

LE factor was present in a high percentage (26.7) of female first degree relatives, the sex difference in frequency of LE factor was less pronounced in second degree relatives. Increased frequency of LE factor by age was very irregular. Younger (less than middle aged) siblings and elderly parents showed the highest figures.

The prevalence of LE factor in the spouses is presented in Table V

The figures were lower than in the relatives, and there was no sex or age difference. LE factor occurred three times more frequently in female relatives than in female spouses. The results of some of the statistical computations of the differences between relatives and spouses are shown in Table VI.

Statistically significant differences were found between female relatives and spouses in the highest age groups, mainly between middle-aged female groups. Significant differences were not observed between male relatives and

Table IV *Indirect LE factor test: Frequency of positive tests in groups of relatives of 72 SLE probands.*

Group	Sex	Age distribution (yr)			Total
		<40	40-59	≥60	
Offspring	female	8 (23.5**)	0		8 (22.2)
	male	1 (2.5)	1 (20.0)		2 (4.5)
Siblings	female	4 (11.7)	11 (33.8)	4 (16.6)	29 (24.1)
	male	0	5 (8.3)	3 (15.0)	8 (7.8)
Parents	female		2 (25.0)	12 (44.4)	14 (40.0)
	male		1 (14.2)	3 (12.0)	4 (12.5)
First degree	female	12 (17.6)	23 (31.9)	18 (31.3)	51 (26.7)
	male	1 (1.6)	7 (9.7)	6 (13.3)	14 (7.8)
	both	13 (10.0)	30 (20.8)	22 (22.9)	65 (17.6)
Sib of mother	female	0	4 (17.3)	9 (20.4)	13 (19.1)
	male	0	2 (8.6)	6 (20.6)	8 (14.5)
Sib of father	female		0	7 (26.9)	7 (18.9)
	male	0	2 (13.3)	4 (15.3)	6 (14.2)
Second degree	female	0	4 (11.7)	16 (22.8)	20 (19.0)
	male	0	4 (10.5)	10 (18.1)	14 (14.4)
	both	0	8 (11.1)	26 (20.8)	34 (16.8)
First and second degree	female	12 (17.3)	27 (25.4)	32 (26.4)	71 (23.9)
	male	1 (1.5)	11 (10.0)	16 (16.0)	28 (10.1)
Other	both	4 (16.6)	0	2 (18.1)	6 (13.6)
Total	both	17 (10.7)	38 (16.8)	50 (21.5)	105 (17.0)

No. of positive in the group

** Percentage of positive in the group

Table V *Indirect LE factor test* Frequency of positive tests in groups of spouses of relatives of 72 SLE probands.

Group	Sex	Age distribution (yrs)			Total
		<40	40-59	>60	
Off-spring	female	0	0		0
	male	0	1 (16.6)		1 (10.0)
Siblings	female	2 (9.0)	4 (10.2)	0	6 (8.6)
	male	1 (5.2)	2 (3.0)	2 (13.3)	5 (5.0)
First degree	female	2 (6.8)	4 (9.7)	0	6 (7.6)
	male	1 (4.3)	3 (4.2)	2 (13.3)	6 (5.5)
	both	3 (5.7)	7 (6.2)	2 (8.6)	12 (6.4)
Sib of mother	female	0	1 (6.2)	0	1 (3.3)
	male		0	2 (6.6)	2 (5.0)
Sib of father	female	1 (50.0)	0	0	1 (3.8)
	male		0	0	0
Second degree	female	1 (25.0)	1 (3.1)	0	2 (3.5)
	male		0	2 (5.2)	2 (3.7)
	both	1 (25.0)	1 (2.1)	2 (3.4)	4 (3.6)
First and second degree	female	3 (9.0)	5 (6.8)	0	8 (5.9)
	male	1 (4.3)	3 (3.4)	4 (7.5)	8 (4.9)
Other	both	2 (25.0)	0	0	2 (11.7)
Total	both	6 (9.3)	8 (4.8)	4 (4.7)	18 (5.7)

The figures are recorded as in Table IV

spouses, but the degree of significance was higher when both sexes were combined. The degree of significance was highest when all age groups were combined and used in the calculations. The difference in occurrence of LE factor in the total populations of relatives and spouses was highly significant.

B. The prevalence of LE factor in rheumatic and non-rheumatic relatives and spouses.

The following variables were considered presence of at least two of ARA criteria for rheumatoid arthritis (RA) (7) effusions in

small (toes and fingers) and large (wrists, elbows, shoulders, knees and ankles) joints, juvenile rheumatoid arthritis, ankylosing spondylitis, Reiter syndrome bursitis, diffuse muscular pains, tenosynovitis, rheumatic fever scleroderma, dermatomyositis, or gout. A non-rheumatic member lacked these variables, a rheumatic member had at least one of the variables.

Relatives. There was a difference in the prevalence of LE factor in rheumatic and non-rheumatic relatives in first degree relatives, LE factor occurred twice as frequently in rheumatic as in non-rheumatic individuals.

Table VI. *Indirect LE factor test* Statistical analysis of the differences between relatives (of 72 SLE probands) and their spouses. Methods: χ^2 calculations and Mantel-Haenszel's method (M-H.) Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups

Group	Sex	Age distribution (yrs)			All age groups combined M-H. two-tailed
		<40 df 1	40 - 59 df 1	>60 df 1	
Siblings	female		0.02		0.0104
First degree	female		0.02		0.0012
	both		0.002		0.0006
Second degree	female				0.025
	male				0.052
	both			0.005	0.002
First and second degree	female		0.003	0.005	<0.0001
	male				0.0854
Total	both		0.0005	0.0008	<0.0001

Rheumatic, male second degree relatives showed high prevalence (26.5 per cent) of LE factor while in non-rheumatic male second degree relatives LE factor was almost absent. Rheumatic female first degree relatives showed no increased frequency of LE factor by age, while non-rheumatic female first degree relatives had an age-dependent frequency.

The results of some of the statistical calculations concerning the difference in prevalence of LE factor between rheumatic and non-rheumatic relatives are shown in Table VII.

Statistically significant differences between rheumatic and non-rheumatic relatives were found in male second degree relatives (mainly due to aged males), middle-aged first degree relatives (mainly due to males), aged second degree relatives and young relatives of the total populations (mainly due to females). When all age groups were combined and used in the calculations, statistically significant differences were also present in the same groups.

Spouses: Rheumatic spouses generally showed higher frequency of LE factor than non-rheumatic spouses, but the differences were small.

Rheumatic relatives and spouses:

The statistical calculations of some of the differences in prevalence of LE factor between rheumatic relatives and spouses are given in Table VIII.

Statistically significant differences were largely found between rheumatic relatives and spouses for the same groups as those recorded in Table VI, but the degree of significance was lower (cf. Tables VI and VIII). The difference of LE factor between rheumatic male first and second degree relatives and spouses was more distinctly present, but still not statistically significant.

Non-rheumatic relatives and spouses still differed significantly in frequency of LE factor.

C. LE factor and size of sibships and families

Two hundred sibships with 571 individuals occurred in first and second degree relatives. The sibship size varied from one to ten when SLE probands were not included. Totally LE factor was found in 34.5 per cent of the sibships, once in 25 per cent, twice in 8.5 per cent, three times in 2.5 per cent and four times

Table VII. *Indirect LE factor test* Statistical analysis of the differences between relatives (of 72 SLE probands) with and without rheumatic disease. Methods: χ^2 calculations and Mantel-Haenszel's method (M-H). Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups.

Group	Sex	Age distribution (yrs)			All age groups combined M-H, two-tailed
		<40 df 1	40 - 59 df 1	>60 df 1	
First degree	female		0.34	0.76	0.09
	male		0.17		
	both		0.0456	0.72	0.0046
Second degree	female			0.43	
	male			0.003	0.003
	both			0.006	0.012
First and second degree	female		0.51	0.48	0.063
	male		0.1	0.16	0.025
Total	both	0.02	0.09	0.106	0.00088

Table VIII. *Indirect LE factor test* Statistical analysis of the differences between relatives (of 72 SLE probands) and their spouses, all presenting evidence of rheumatic disease. Methods: χ^2 calculations and Mantel-Haenszel's method (M-H). Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups.

Group	Sex	Age distribution (yrs)			All age groups combined M-H, two-tailed
		<40 df 1	40 - 59 df 1	>60 df 1	
First degree	female		0.17		0.12
	male				
	both		0.024		0.028
First and second degree	female		0.04	0.03	0.003
	male		0.17	0.31	0.056
Total	both		0.012	0.008	0.0002

In a single sibship in a sibship of three all members presented LE factor

The family size varied from eight to eighteen when the SLE probands were not included. LE factor was present in two-thirds of the SLE families, one in 7.7 per cent, twice in 19.5 per cent, three times in 1.5 per cent, four times in 4.4 per cent and five times in 1.4 per cent.

In a family of sixteen seven members presented LE factor.

There was no apparent increase of LE factor by sibship or family size and the aggregation of LE factor in relatives was not due to an accumulation of the factor in a minority of sibships or families. The distribution of LE factor in sibships and families was calculated

and f und consistent with the Poisson distribution.

D. LE factor and the residence of relatives

One-fifth of the relatives lived outside the defined area in central eastern Norway in which the SLE probands were diagnosed (19). The prevalence of LE factor did not differ in relatives and controls living outside or inside this area.

E. Genetic basis for LE factor

A study of the genetic basis of the occurrence of an antibody is difficult, as the presence of an antibody requires both the gene(s) and an adequate stimulus, and the stimulus for the induction of LE factor is unknown.

The offspring of parents without LE factor often exhibited the factor. It thus appears unlikely that the occurrence of LE factor is due to single dominant gene.

A method for checking the agreement of the observed segregation of a character in family data, and the expected segregations calculated on the basis of Mendelian ratios — χ^2 test for the simple case of two alleles, one recessive to the other — has been evolved by Smith (26, 27). Smith's method was used for LE factor as character in the present family material, but the results of the tests for agreement between observed and expected numbers of (I) recessives (with negative LE factor) and of (II) affected (with positive LE factor) make it unlikely that the occurrence of LE factor is due to monofactorial dominant or recessive inheritance.

As previously stated, the familial aggregation of LE factor was not related to sibship or family size, nor was it due to accumulation of LE factor in minority of sibships. A high prevalence of condition (here LE factor) both in first and second degree relatives is suggestive of a multifactorial (genetic) influence and Falconer has developed a method of assessing the likelihood of an individual developing the same multifactorially determined disease as relative (for references, see 6). If we assume that the prevalence of LE factor is similar in spouses and in general population, the heritability of the LE factor in first degree relatives

was found to be about 80 per cent, using Falconer's formulae. The prevalence of LE factor was 17.6 and 6.4 per cent in first degree relatives and spouses, respectively. However, the prevalence of LE factor in second degree relatives and spouses was even higher (16.8 per cent and 3.6 per cent, respectively) and as the coefficient of relationship for second degree relatives is 1/4, the approximate estimate of the heritability of LE factor from the present family sample becomes too high. This is probably partly due to the selection of SLE probands, as numerous LE cells is only one of the criteria upon which the diagnosis of SLE was based (19). The frequency of LE factor is also age- and sex-dependent, which makes the calculation of heritability even more complicated (11).

Tests for penetrance were not performed.

DISCUSSION

LE factor was originally defined as factor that induced the morphologically typical LE cell phenomenon (15); here, LE factor denotes a factor giving any of the various morphological patterns (homogeneous and large round or amorphous LE bodies (globes) and rosettes) usually connected with true LE cells (1, 13, 30). The basis for this concept is given in detail in the paper describing the indirect LE factor test (18).

There are very few reports about the occurrence of LE factor in families of patients with SLE (25, 33, 34). This is usually due to lack of suitable techniques for demonstration of LE factor in population studies and to lack of adequate criteria for weakly positive LE factor tests.

The prevalence of LE factor was determined in 615 relatives, and their 313 spouses, of 72 SLE probands from central eastern Norway by newly-developed, simple sensitive and standardized indirect LE factor test (18). There was significant increase of LE factor in relatives compared with their spouses. All groups of relatives showed increased values, but the familial aggregation was most pronounced in female subjects, the sex difference in frequency of LE factor being very marked. Younger offspring,

middle-aged siblings and elderly parents showed the highest figures in first degree and elderly parents showed the highest figures; in first degree relatives, the frequency of LE factor was rather constant after forty years of age. In second degree relatives, the frequency of LE factor increased gradually by age. Familial accumulation of LE factor occurred both on the maternal and paternal sides and with similar frequency.

Antinuclear factors (ANFs) detected by immunofluorescence using rat liver as source of nuclei, also showed familial aggregation in the present material (29). In some groups of relatives, the frequency of LE factor exceeded the frequency of ANFs. It is usually stated that sera giving positive LE cell phenomenon and no sign of ANFs by immunofluorescence occur very rarely and when present the phenomenon is said to be due to altered antigen in the former test (31). The higher frequency of LE factor in some groups of relatives in the present material may be due to different sources of nuclei in the two tests and to the interpretation of positive LE cell tests. Granulocyte-specific ANFs occur in sera and synovial fluids of patients with rheumatoid arthritis (10). Granulocyte-specific LE factor was demonstrated in sera of patients with systemic lupus erythematosus (18). Nuclei of granulocytes are the main source of antigen in the indirect LE factor test (18). This favours demonstration of granulocyte-specific LE factor and might thus explain some of the cases with positive LE factor test and no ANFs detectable by the immunofluorescence test. Generally sera containing LE factor give peripheral or homogeneous nuclear staining in immunofluorescence tests for ANFs (14). In the present study LE factor and homogeneous ANF were better correlated in relatives than in spouses (29).

The prevalence of LE factor in sera of relatives was not correlated to the size of sibships or families, as was the case for rheumatoid factor in other population studies (24). The number of members with LE factor increased gradually with increasing family size.

Presence of LE factor in sera of relatives was partly related to rheumatic disease. Rheumatic relatives and spouses still differed significantly in frequency of LE factor in contrast to the findings with regard to ANF in the same

populations (29). Female rheumatic and non-rheumatic relatives do not differ while the male populations behave very differently.

The cause of familial aggregation of LE factor and most other auto-antibodies is still obscure. The occurrence of rheumatoid factors (RFs) and ANFs in relatives of SLE probands has been interpreted as evidence for an inherited abnormality of the immune system (30). However a genetic study of rheumatoid arthritis (RA) and RF in Blackfeet and Pima Indians revealed no evidence that genetic factors played an important part in the etiology of RA and RF in these tribes (24). Both the studies in Indian tribes and in twins indicated that environmental factors are of prime importance in the development of auto-antibodies, at least in apparently healthy individuals (3).

The presence of ANFs and LE factor in inbred strains of mice (22) probably parallels the observations made in families of patients with SLE. Although the mice are known to be infected with virus (21) experiments with hybrids stress the importance of genetic factors (4, 12, 17) for the occurrence of serological abnormalities and auto-antibodies.

The findings in the present family study do not fit any simple type of inheritance. The increased, but irregular occurrence of LE factor in families of patients with SLE points to a multifactorial etiology of the LE factor where environmental factors probably play an important role. Spouses of relatives were used as controls to reduce the importance of environmental factors. Although most family members had lived some distance from each other for many years, this did not affect the prevalence of LE factor. It was difficult to achieve estimates of heritability of LE factor in the present family material when Falconer's method (6) including both environmental and genetic factors was used. The present material does not permit any conclusions as to the relative importance of genetic and environmental factors and the genetic mechanism involved in the aggregation of LE factor in families of patients with SLE, to solve this problem more information is needed on the mechanism of induction of LE factor.

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IV Presence of antinuclear factors (ANFs) in the total populations of relatives and spouses, and the correlation to rheumatic disease

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ABSTRACT

The distribution of antinuclear factors in sera of 615 relatives, and their 513 spouses, of 72 SLE probands is presented. A statistically significant, but irregular aggregation of ANF occurred in relatives. When the different patterns of nuclear fluorescence were analysed, the homogeneous type, but not the speckled type of ANF separated the relatives and the spouses. The correlation between ANF and rheumatic disease was more manifest for relatives than for spouses. Non-rheumatic relatives and spouses did not differ with regard to any type of ANF.

Immunofluorescent techniques for demonstration of antinuclear factors (ANF) in SLE and allied disorders were described by separate research groups in 1957 (9, 10, 11, 18, 24). ANF are also present in non-rheumatic diseases (3) and in healthy individuals (4, 13). ANFs react with different nuclear antigens (2, 12, 25) and belong to different classes of immunoglobulins (1).

ANFs occur with increased frequency in relatives of patients with SLE, but the prevalence of ANF in the families varies greatly (for references, see 7, 17). This is partly due to variations in the techniques used, but different selection of SLE probands may also contribute to the variation. There are still no definite generally accepted criteria for the diagnosis of SLE (20).

The present work is part of an extensive investigation of clinical and serological aberrations in relatives of SLE probands from central eastern Norway. This paper deals with ANF in the total populations of relatives and their spouses and the correlation to rheumatic disease. In the accompanying paper (16) the

occurrence of ANF in various populations of relatives of selected SLE probands is described.

MATERIALS AND METHODS

SLE probands: The criteria for selection of the 72 probands with SLE and their clinical data have been published elsewhere (14). The probands represented all individuals considered to be definite SLE cases in a defined area from central eastern Norway during the years 1959–1963. Fifty-one probands with SLE were available for further blood studies, but the disease was not necessarily active during blood sampling.

Relatives and their spouses: The sampling of relatives and spouses used as controls, as well as the age and sex distribution of the clinically and serologically examined relatives and spouses, have also been reported elsewhere (15). First degree relatives (children, siblings and parents), second degree relatives (siblings of parents) and spouses were primarily included in the investigation. «Other relatives» mainly

grandparents and grandchildren, were also included when available. The completion rates of the various groups of relatives varied from 86.6 to 98.5 per cent. The completion rates of the various groups of spouses varied from 82.4 to 91.4 per cent.

Statistical methods: The data were punched on data cards and processed in a CD 3300 computer at the University of Oslo Norway. The age distribution usually differed in the various populations. ANF positivity is regarded as being age-dependent (3, 4, 23). χ^2 calculations were therefore performed separately for each age group and the total group (obtained by summing up the individuals in the various age groups) but significant differences (p values below 0.05) between total groups were usually not taken into account. When the task was to combine evidence for the three age groups (a number of 2×2 tables) Mantel-Haenszel's method (M-H) was employed (22). A continuity correction was used (8, 22).

All p-values of significant level (< 0.05) are given in the tables. However when the p-values in specified groups seem to contribute to significant differences when groups are summarized, non-significant p-values are also indicated. In some instances, non-significant p-values are also given.

Tests for ANFs.

(1) **Sera.** Sera of probands, relatives and spouses were stored in small aliquots at 22°C and thawed once before use. The sera were coded and tested by a double blind technique.

(2) **Conjugates.** Rabbit antisera against pepson-split human IgG (19) were used to ensure demonstration of ANF of all immunoglobulin classes. The antisera were made 1.65 M in $(\text{NH}_4)_2\text{SO}_4$. The precipitates were washed and then dissolved in distilled water of half the original volume of serum, and dialysed extensively against saline. Conjugation with fluorescein-isothiocyanate (FITC) was performed using 15 mg FITC per g protein in carbonate bicarbonate buffer pH 9.0 at 4°C overnight. The conjugated FITC was moved by gel filtration on Sephadex G. The conjugate was diluted approximately 10 times the original serum volume. The dilution consistently gave good homogeneity and reacted with the ANF serum diluted 1:4000. Controls for unspecific staining were prepared regularly and did not show absorption of

the conjugates was unnecessary.

(3) **Buffers:** Phosphate buffer (0.15 M KH_2PO_4 and 0.15 M $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) of pH 7.0 was used. For washing procedures and dilution of the sera phosphate buffered saline (1 part phosphate buffer and 9 parts 0.9 per cent NaCl) was used.

(4) **Source of nuclei, incubation and microscopy.** Unfixed cryostat sections (5–10 μ thick) of fresh snap-frozen rat liver were air-dried at room temperature for 10–30 minutes, incubated with serum (undiluted and diluted 1/16) for 30 minutes, washed 3 times for 5 minutes by gentle agitation, incubated with conjugate for 30 minutes, washed 3 times for 5 minutes, and finally mounted with buffered (pH 7.0) glycerol-gelatin. Incubation and washing were performed at room temperature and all tests were made in duplicate. Positive and negative controls were included in each series. The sections were examined the same day by dark field ultraviolet light using a Zeiss microscope (primary filter Scott BG 12 and secondary filter 41–65 or 44–63).

Three distinct patterns of nuclear fluorescence were observed: (2) homogeneous speckled and nucleolar. Sera which showed nuclear fluorescence when diluted 1/16 were set up in twofold serial dilutions, and the last dilution which gave a weak but distinct fluorescence determined the titre. These tests were also performed in duplicate. The degree of nuclear fluorescence was graded from + to +++++; one plus fluorescence was regarded as weak, and +++ and +++++ as strong fluorescence. Sera with titres of 1/16 and higher were considered as positive. ANFs were classified according to the patterns of nuclear fluorescence. The sera giving more than one pattern were classified according to the different patterns. Thus, the number of positive tests were grouped as follows:

(1) All patterns combined, i.e., all individuals with at least one pattern present. Nucleolar pattern was extremely rare and was therefore included in this group. (2) Homogeneous pattern, i.e., all individuals with homogeneous pattern even if other patterns were present. (3) Speckled pattern, i.e., all individuals with speckled pattern even if other patterns were present.

The tests were performed during the years 1964–1966.

RESULTS

A. The prevalence of ANFs in SLE probands

ANFs (all patterns combined) were demonstrated in 37 (72.3 per cent) of the 51 SLE probands tested. Homogeneous and speckled ANF were found in 58.8 and 33.3 per cent respectively. Nucleolar pattern was rare and only observed in 3 patients.

B. The prevalence of ANF in relatives and spouses.

(1) *All patterns combined* Sera of 20.9 per cent of the relatives and 13.7 per cent of the spouses were positive. The age and sex distribution of ANF in relatives and spouses is presented in Tables I and II.

Table I. ANFs determined by immunofluorescence. Frequency of positive tests (all patterns combined) in groups of relatives of 72 SLE probands.

Group	Sex	Age distribution (yrs)			Total
		<40	40 - 59	≥60	
Offspring	female	6 (17.6 ^{ab})	0		6 (16.6)
	male	5 (12.8)	2 (40.0)		7 (15.9)
Siblings	female	4 (11.7)	17 (27.4)	5 (20.8)	26 (22.8)
	male	3 (13.6)	12 (20.0)	2 (10.0)	17 (26.6)
Parents	female		8	8 (29.6)	8 (22.8)
	male		2 (28.5)	9 (36.0)	11 (34.3)
First degree	female	10 (14.7)	17 (23.6)	13 (25.4)	40 (20.9)
	male	8 (13.1)	16 (22.2)	11 (24.4)	35 (19.6)
	both	18 (13.9)	33 (22.9)	24 (25.0)	75 (20.3)
Sib. of mother	female	0	4 (17.3)	9 (20.4)	13 (19.1)
	male	0	3 (13.0)	5 (17.2)	8 (14.5)
Sib. of father	female		3 (27.2)	11 (42.3)	14 (37.8)
	male	0	4 (26.6)	6 (23.0)	10 (23.8)
Second degree	female	0	7 (20.5)	20 (28.5)	27 (25.7)
	male	0	7 (18.4)	11 (20.0)	18 (18.5)
	both	0	14 (19.4)	31 (24.8)	45 (22.2)
First and second degree	female	10 (14.4)	24 (22.6)	33 (27.3)	67 (22.6)
	male	8 (12.3)	23 (20.9)	22 (22.0)	53 (19.2)
Other	both	5 (20.8)	1 (11.1)	3 (27.2)	9 (20.4)
Total	both	23 (14.5)	48 (21.3)	58 (25.0)	129 (20.9)

^a No. of positive in the group
^b Percentage of positive in the group

Table VII ANF determined by immunofluorescence. Statistical analysis of the difference between relatives (of 72 SLE probands) with and without rheumatoid symptoms. Methods: χ^2 -calculations and Mantel-Haenszel's method (M-H.). Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups.

Group	Sex	ANF pattern	Age distribution (yrs)			All age groups combined M-H. two-tailed
			<40 df 1	40-59 df 1	≥60 df 1	
First degree	male	all comb.		0.20		0.082
	both	homogen.		0.406	0.79	
	male	speckled		0.31		0.064
	both	speckled		0.406	0.63	0.117
Second degree	both	all comb.			0.067	
	both	homogen.		0.97	0.026	0.0387
	both	speckled		0.79	0.42	0.31
First and second degree	male	all comb.		0.34	0.566	0.039
	female	homogen.		0.828	0.163	
	male	homogen.		0.206		0.151
	female	speckled		0.56	0.29	
	male	speckled		0.465	0.767	0.064
Total	both	all comb.		0.67	0.19	0.20
	both	homogen.		0.48	0.185	0.069
	both	speckled		0.31	0.36	0.089

cent, respectively) male individuals showing highest differences. A statistically significant difference between rheumatic and non-rheumatic relatives was present for aged second degree relatives and total second degree relatives when all age groups were used. The difference between the total populations of relatives was not statistically significant (cf Table VII).

(3) Speckled pattern. This type of ANF and rheumatic variables showed a slight association. Rheumatic male first degree relatives had this type of ANF in 1 per cent compared with 9.8 per cent in rheumatic first degree males. However, the difference between the two populations of relatives was not statistically significant.

Spouse

Rheumatic and non-rheumatic spouses did not differ in ANF.

Relatives and spouses with rheumatic diseases

Sera of rheumatic relatives contained ANFs more frequently than sera of rheumatic spouses, the degree of significance of the differences between rheumatic relatives and spouses was generally higher than for the total populations of relatives and spouses, mainly due to first degree males and second degree females, when all patterns were combined, and to second degree females when homogeneous ANF was considered. The results of some of the statistical calculations are shown in Table VIII. The frequency of ANF giving the speckled pattern did not differ significantly between rheumatic relatives and spouses.

Relatives and spouses without rheumatic disease

Non-rheumatic relatives and spouses did not differ with regard to any type of ANF.

Table VIII. ANF determined by immunofluorescence. Statistical analysis of the differences between relatives (of 72 SLE probands) and their spouses, all presenting evidence of rheumatic disease. Methods: χ^2 calculations and Mantel-Haenszel' method (M-H.) Degrees of freedom (df) are indicated. The figures show the p-values in the different age and sex groups.

Group	Sex	ANF pattern	Age distribution (yrs)			All age groups combined M-H. two-tailed
			<40 df 1	40 - 59 df 1	>60 df 1	
First degree	female	all comb				
	male	all comb		0.115		0.054
	both	all comb		0.0957		
	female	homogen.		0.49		
	male	homogen.		0.17		
	both	homogen.		0.08	0.38	
Second degree	female	all comb				0.0377
	male	all comb				0.257
	both	all comb			0.048	0.01
	female	homogen.				0.017
	male	homogen.				
	both	homogen.		0.25	0.06	0.0157
First and second degree	female	all comb		0.19	0.089	
	male	all comb		0.098	0.38	0.0227
	female	homogen.		0.126	0.054	
	male	homogen.		0.169		0.099
Total	both	all comb		0.0257	0.0246	0.0058
	both	homogen.		0.029	0.024	0.0018

D. The correlation between LE factor and ANF in relatives and spouses.

The prevalence of LE factor in relatives and spouses has been reported elsewhere (14). Fifty-two (69.5 per cent) out of 75 relatives with homogeneous ANF had LE factor; the percentage in spouses was 37.5 per cent. The correlation was highest in female first degree relatives (95.7 per cent). LE factor and speckled ANF were not related, the percentages in relatives and spouses being 31.7 and 22.9 respectively.

E. ANF and size of sibships and families.

Two hundred sibships with 571 individuals

occurred in first and second degree relatives. The sibship size varied from one to ten when the SLE probands were not considered. No apparent increase of ANF by size of sibships was noticed, e.g. homogeneous ANF was present in 28.5 per cent of the sibships: once in 23 per cent, twice in 4.5 per cent, three times in 0.5 per cent and four times in 0.5 per cent. In one sibship of eight four members presented homogeneous ANF.

The family size, including first and second degree relatives, varied from one to eighteen when the SLE probands were not considered. No apparent increase of ANF by size of families was noticed, e.g., homogeneous ANF was found in 58.3 per cent of the families: once

in 40.1 per cent, twice in 4.2 per cent, three times in 9.7 per cent, four times in 1.4 per cent, etc. In one family of sixteen, six members presented homogeneous ANF.

The distribution of ANFs in sibships and families was calculated and found consistent with the Poisson distribution.

ANFs and the residence of relatives and spouses.

One fifth of the relatives and spouses lived outside the area in which the SLE probands were diagnosed (15). The prevalence of ANFs did not differ in relatives and in controls living outside and inside this area.

DISCUSSION

A negative ANF test usually makes the diagnosis of definite SLE unlikely (for references, see 5). In the present material, ANFs were demonstrated in 57 (72.5 per cent) of the 81 SLE probands tested. However those who had negative ANF tests exhibited no disease activity when the blood samples were taken. We regarded this to be the explanation as the presence of antinuclear antibodies, especially anti-DNA, and other antibodies in SLE is correlated to disease activity (21).

The prevalence of ANFs was determined in 615 relatives, and their 513 spouses, of 72 SLE probands from central eastern Norway. Three distinct patterns of nuclear fluorescence (homogeneous, speckled and nucleolar) were observed, but the nucleolar pattern was rare.

We have demonstrated an aggregation of ANFs in the relatives of the SLE probands when compared with their spouses who served as controls, but the distribution of the different types of ANF was very irregular. As a rule ANF occurred more frequently in all groups of relatives above forty years of age. Age-dependent frequency of ANF is even more pronounced in normal type (4) and then mostly in females (5, 3).

Female relatives showed the highest frequencies of ANF, but the difference was moderate and irregular. The speckled type was most frequent, both in

with the exception of young females (highest frequency in spouses) there was no significant difference between relatives and spouses. By contrast, ANF giving the homogeneous pattern clearly separated the two populations, mainly due to females. Male relatives did not differ significantly from male spouses.

The relatives and the spouses differed in other ways, the correlation between ANF and rheumatic disease was more conspicuous for relatives than for spouses. Rheumatic and non-rheumatic relatives generally differed in ANF most distinctly for young male first degree relatives (speckled type) and second degree relatives (homogeneous type). By contrast, rheumatic and non-rheumatic spouses did not differ in any type of ANF. This was also noticed for non-rheumatic relatives and spouses. LE factor and homogeneous ANF were better correlated in relatives than in spouses.

The cause of the irregular aggregation of ANFs in relatives of SLE probands is obscure. There was no increase of ANFs with sibship or family size and ANFs occurred with similar frequencies in family members who had lived far from each other for many years. The irregular aggregation of ANFs in relatives may indicate that different populations of relatives with varying prevalence of ANFs actually occur. Different clinical symptoms may predominate during active disease in individual patients with SLE. We have therefore selected SLE probands on clinical, serological, or combined clinical-serological basis; the results of tests for ANF in the various populations of relatives of these selected SLE probands will be presented in the accompanying paper.

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V Presence of antinuclear factors (ANFs) in and spouses of selected SLE probands

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ABSTRACT

Antinuclear factors (ANF) were studied in sera of 81 SLE probands, 615 relatives and their 313 spouses who served as controls.

The frequency of ANF in the relatives was correlated to certain variables present in the SLE probands. A very marked accumulation of speckled ANF was found in relatives of SLE probands with marked arthritis, whose female relatives above forty years of age more frequently had a present or past history of effusions in large joints than female relatives of SLE probands without arthritis. The frequency of ANF in relatives was also correlated with in the titer of ANF in the proband and with in the presence of numerous LE cells at the time of diagnosis of SLE in the proband.

Selection of subgroups of SLE probands with defined clinical and/or serological characteristics appears to be valuable in family studies of SLE.

The prevalence of antinuclear factors (ANFs) is increased in relatives of patients with SLE (for references, see 5, 16). Varying frequencies in the different family materials are probably due to variations in the immunofluorescent methods used and in the selection of probands. We have confirmed the aggregation of ANFs in relatives of SLE probands in Norwegian material (18).

The distribution of antinuclear factors in the relatives was irregular. This may indicate that different populations of relatives with varying frequency of ANF actually occur. Different clinical symptoms may predominate during active disease in individual patients with SLE (13) and definite criteria for the diagnosis of SLE have not yet been established (17). We have therefore selected SLE probands on a clinical, serological, or combined clinical-serological basis. The corresponding population of relatives showed marked differences in occurrence of ANFs. The results of these studies are presented in this paper.

MATERIALS AND METHODS

SLE probands, relatives and spouses. The criteria for selection of the probands with SLE, the mode of collecting sera and clinical data, the age and sex distribution, and the complication rates have been reported elsewhere (13, 14).

Statistical methods: The methods used have been described previously (18).

Tests for ANFs: The immunofluorescent method for demonstration of antinuclear factors and the criteria for positive reactions have been described in detail in the preceding paper (18).

Principles of selection of subgroups of SLE probands: The subgroups of the SLE probands were selected on the basis of the following characteristics: presence of lupus nephritis, hypergammaglobulinemia, acute or chronic lupus skin lesions, numerous LE cells at the time of diagnosis, severe arthritis, and wire-loops (13). Fifty-one SLE probands were also classified according to the titers of ANFs. Finally

combined clinical characteristics and titer of ANFs were also used as criteria.

Comparison was first made between the frequency of ANFs in the relatives of SLE probands with and without certain feature. This was followed by comparison of the frequency in the corresponding spouses who served as controls. In the subsequent text, only those subgroups of SLE probands are described in which the corresponding relatives showed a significant increase of ANFs.

RESULTS

A. Variable for selection. Marked arthritis in the SLE proband.

SLE probands were classified according to ARA's criteria for rheumatoid arthritis (RA) (4) SLE probands who developed RA features during their disease manifested these properties very early and initially showed a high incidence of effusions in small (finger and toe) and large (wrist elbow shoulder knee and ankle) joints (13) Twenty-four (53.3 per cent) of the SLE probands showed predominating symptoms of arthritis.

ANFs in relatives:

The proportion of relatives of the two groups of SLE probands was 1:2.

(1) All patterns combined: ANFs were demonstrated in 27.2 per cent of first degree relatives of SLE probands with marked arthritis and in 16.9 per cent of first degree relatives of probands without arthritis. A similar difference was found between second degree relatives. The distribution is seen in Table I

Some of the results of the statistical calculations are presented in Table II.

The relatives of SLE probands with marked arthritis more frequently had ANFs. Statistically significant differences between the two types of relatives were found for aged females. The degree of significance was higher when all age groups or first and second degree relatives were combined and used in the calculations. Significant differences were still present when both sexes were combined.

(2) Homogeneous pattern. Sera giving homogeneous nuclear pattern were equally distributed in the two populations of relatives.

(3) Speckled pattern. Sera of female relatives of SLE probands with marked arthritis gave speckled nuclear fluorescence about four times as often as the sera of female relatives of SLE probands without arthritis. There was a female preponderance of speckled ANF in relatives of SLE probands with arthritis, but the sex distribution of positive tests was reversed in relatives of SLE probands without arthritis. There was no marked age variation of speckled ANF in the populations. The distribution is shown in Table III

The results of statistical calculations are shown in Table IV

Statistically significant differences between the two groups of relatives were seen in the highest age groups and when all age groups were combined. The differences were mainly due to female first degree relatives. However the degree of significance was higher when first and second degree relatives were combined. When both sexes were combined, the significant differences were still present. The difference of speckled ANF in the total populations of relatives was highly significant.

ANFs in spouses.

We did not record any differences in frequency of ANFs in the corresponding two populations of spouses.

Clinical variables in relatives:

The frequency of ARA's criteria for RA and of joint effusions in female relatives is shown in Table V

Male relatives did not differ. A past or present history of effusions in large joints occurred significantly more frequently in female relatives of SLE probands with marked arthritis than in female relatives of SLE probands without arthritis (using χ^2 -calculations, $p < 0.005$ df 1). The frequency of effusions in large joints in the corresponding two populations of spouses was not different. The distribution of sera giving homogeneous nuclear fluorescence in the two populations of female relatives with effusions in joints was different. About half of the sera of female arthritic relatives of SLE probands with arthritis, versus only 11 per cent of female arthritic relatives of SLE probands without arthritis showed homogeneous nuclear fluorescence. Speckled ANF occurred irregularly and was not related to joint effusions in female relatives.

Table 1 ANFs determined by immunofluorescence. Frequency of positive tests (all patterns combined) in relatives of SLE probands with and without marked arthritis.

Arthritis in SLE probands	Group of relatives	Sex	Age distribution (yrs)			Total
			<40	40-59	≥ 60	
Present	Siblings	female	1 (14.2 ^{ab})	9 (31.0)	3 (25.0)	13 (27.0)
Absent		female	3 (11.1)	8 (24.2)	2 (16.6)	13 (18.0)
Present	First degree	female	3 (16.6)	9 (30.0)	9 (40.9)	21 (30.0)
		male	1 (7.6)	6 (26.0)	5 (33.3)	12 (23.5)
		both	4 (12.9)	15 (28.3)	14 (37.8)	33 (27.2)
Absent	First degree	female	7 (14.0)	8 (19.0)	4 (13.7)	19 (15.7)
		male	7 (14.5)	10 (20.4)	6 (20.0)	23 (18.1)
		both	14 (14.2)	18 (19.7)	10 (16.9)	42 (16.9)
Present	Second degree	female		1 (33.3)	10 (47.6)	11 (45.8)
		male	0	2 (28.5)	5 (23.8)	7 (24.1)
		both	0	3 (30.0)	15 (35.7)	18 (33.9)
Absent	Second degree	female	0	6 (19.3)	10 (20.4)	16 (19.7)
		male	0	5 (16.1)	6 (17.6)	11 (16.1)
		both	0	11 (17.7)	16 (19.2)	27 (18.1)
Present	First and second degree	female	3 (16.6)	10 (30.3)	18 (44.1)	32 (34.0)
		male	1 (7.1)	8 (26.6)	10 (27.7)	19 (23.7)
Absent	First and second degree	female	7 (13.7)	14 (19.1)	14 (17.9)	35 (17.3)
		male	7 (13.7)	15 (18.7)	12 (18.7)	34 (17.4)
Present	Total	both	7 (15.5)	18 (26.0)	30 (34.8)	55 (27.5)
Absent		both	16 (14.1)	30 (19.2)	28 (19.1)	74 (17.8)

No. of positive in the group

^{ab} Percentage of positive in the group

B. Variables for selection: High-titered (titer > 1/256) versus negative tests for particular type of ANF in the SLE probands

The families of these two categories of SLE probands were compared to investigate whether the pattern of ANF in the SLE probands and the relatives were correlated.

(1) All patterns combined

Seventeen SLE probands had high titers of this type of ANF and fourteen SLE probands were negative.

Relatives: One hundred and fifty-five and 102 relatives belonged to the two populations, i.e. relatives of high-titered and negative pro-

Table IV ANFs giving a speckled pattern in immunofluorescence Statistical analysis of the differences between relatives of SLE probands with and without marked arthritis. Methods: χ^2 calculations and Mantel-Haenszel's method (M-H.) Degrees of freedom (df) are indicated. The figures show the p-values in the different age and sex groups.

Group	Sex	Age distribution (yrs)			All age groups combined M-H, two-tailed
		<40 df 1	40-59 df 1	≥60 df 1	
Siblings	female		0.044		0.06
First degree	female		0.047	0.11	0.001
	male				
	both		0.04	0.03	0.002
Second degree	female			0.16	0.08
	male				
	both		0.2	0.2	0.046
First and second degree	female		0.013	0.015	<0.0001
	male			0.32	0.2
Total	both		0.024	0.02	0.0006

Table V Rheumatoid symptoms (a past or present history) in female relatives of SLE probands with and without marked arthritis.

Arthritis in SLE proband	Symptoms in relatives	Years of age		
		<40	≥40	Total
present	2	2 (6.8 ⁰⁰)	7 (8.1)	9 (7.8)
absent		4 (6.9)	12 (7.5)	16 (7.4)
present	No. of ARA criteria for RA	2 (6.8)	10 (11.6)	12 (10.4)
absent		0	13 (8.2)	13 (6.0)
present	5-6	0	5 (5.8)	5 (4.3)
absent		0	4 (2.5)	4 (1.9)
present	small joints	0	8 (9.3)	8 (7.0)
absent		0	9 (5.7)	9 (4.2)
present	Effusions in large joints	2 (6.8)	11 (12.8)	13 (11.3)
absent		0	6 (3.7)	6 (2.8)

No. of affected individuals in the group

⁰⁰ Percentage of affected individual in the group

Table VI. ANFs determined by immunofluorescence: Frequency of positive tests in relatives of SLE probands with high titres of ANF and negative tests for ANFs.

ANFs in SLE probands	Group of relatives	Sex	Age distribution (yrs)			Total
			<40	40-59	≥60	
high titer	Second degree	female		2 (100)	11 (42.3)	13 (46.4)
		both	0	4 (80.0)	14 (33.3)	18 (38.2)
negative	Second degree	female		0	1 (7.6)	1 (6.6)
		both		1 (14.2)	3 (13.6)	4 (13.7)
high titer	First and second degree	female	2 (12.5)	8 (33.3)	18 (42.8)	28 (33.3)
negative	Second degree	female	2 (18.1)	4 (25.0)	3 (13.0)	9 (18.0)

The figures are recorded as in Table I.

Table VII. ANFs determined by immunofluorescence: Frequency of positive tests in relatives of SLE probands with numerous or few LE cells at the time of SLE diagnosis.

LE cells in SLE probands	Group of relatives	Sex	Age distribution (yrs)			Total
			<40	40-59	≥60	
numerous	First degree	female	8 (19.5)	14 (25.9)	8 (24.2)	30 (23.4)
		male	6 (15.3)	15 (28.3)	9 (28.1)	30 (24.1)
		both	14 (17.5)	29 (27.1)	17 (26.1)	60 (23.8)
few	First degree	female	2 (8.0)	3 (16.6)	4 (23.5)	9 (15.0)
		male	2 (9.5)	1 (5.2)	1 (8.3)	4 (7.6)
		both	4 (8.6)	4 (10.8)	5 (17.2)	13 (11.6)
numerous	First and second degree	female	8 (19.5)	20 (25.6)	26 (29.5)	54 (26.0)
		male	6 (14.2)	18 (23.6)	16 (22.2)	40 (21.0)
		both	14 (16.6)	38 (33.9)	42 (30.8)	94 (33.2)
few	First and second degree	female	2 (7.6)	4 (14.2)	6 (20.0)	12 (14.2)
		male	2 (9.0)	5 (14.7)	4 (15.3)	11 (13.4)
		both	4 (11.3)	9 (14.5)	10 (17.5)	23 (14.5)

The figures are recorded as in Table I.

Table VIII. ANFs giving a speckled pattern in immunofluorescence. Frequency of positive tests in relatives of SLE probands with numerous or few LE cells at the time of SLE diagnosis.

LE cells in SLE probands	Group of relatives	Sex	Age distribution (yrs)			T test
			<40	40-59	≥60	
numerous		female	4 (9.7)	8 (14.8)	5 (15.1)	17 (13.2)
		male	6 (15.3)	11 (20.7)	6 (18.7)	23 (18.5)
		both	10 (12.5)	19 (17.7)	11 (16.9)	40 (15.8)
few	First degree	female	1 (4.0)	1 (5.5)	2 (11.7)	4 (6.6)
		male	2 (9.5)	0	1 (8.3)	3 (5.7)
		both	3 (6.5)	1 (2.7)	3 (10.3)	7 (6.2)

The figures are recorded as in Table I

was not tested for LE-cells at the time of diagnosis, and had to be excluded.

Relatives

Four hundred and thirty-five and 172 relatives corresponded to the two categories of SLE probands.

(1) All patterns combined: The proportion of positive tests for ANF was 2.1 in the two populations. The distribution is presented in Table VII.

Some of the statistical calculations are shown in Table VIII.

A significant difference was found for male first degree relatives when all age groups were used in the calculations, but the degree of significance was higher for all first degree relatives. The difference in female first and second degree relatives was not significant. However the difference between the total populations of relatives was statistically significant.

(2) Homogeneous pattern: The distribution of this type of ANF was very irregular with no statistically significant differences between the populations (cf. Table VIII).

(3) Speckled pattern: The proportion of speckled ANF in the two populations of relatives was 1. The distribution in first degree relatives is shown in Table IX.

The statistical calculations are included in Table VIII. Statistically significant differences were found between middle-aged first degree relatives, and between first degree relatives

when all age groups were combined and used in the calculations.

Spouses

No statistically significant differences were observed.

DISCUSSION

We observed that the accumulation of ANFs in the relatives of probands with SLE was correlated to clinical and serological findings in the probands.

Speckled ANF appeared to be nearly equally distributed in relatives and spouses of the total proband material (18) but sera of relatives of SLE probands with and without marked arthritis gave different fluorescence. Speckled antinuclear factor occurred more frequently in relatives of SLE probands with arthritis, the aggregation was statistically significant both in first and second degree relatives, and was mainly due to females.

Speckled ANF is known to react with phosphate buffer extract of nuclei (12) and occurs in SLE and allied syndromes (7). The pattern of nuclear staining is more obvious in tumour imprints, and so-called true speckled staining is present in scleroderma and Raynaud phenomena (3). Raynaud phenomena occurred with increased frequency in the SLE probands with marked arthritis (13) and it is possible

Table IX. ANFs determined by immunofluorescence. Statistical analysis of the difference between relatives of SLE probands with numerous or few LE cells at the time of SLE diagnosis. Methods: χ^2 calculations and Mantel-Haenszel method (M-H). Degrees of freedom (df) are indicated. The figures show the p-values in different age and sex groups.

Group	Sex	ANF pattern	Age distribution (yrs)			All age groups combined M-H, two-tailed
			<40 df 1	40-59 df 1	>60 df 1	
First degree	male	Speckled		0.074		0.056
	male	All comb		0.079		0.027
	both	Speckled		0.045		0.028
	both	All comb		0.07		0.017
Second degree	female	Homogen.				0.087
	female	All comb				
First and second degree	female	Homogen.				0.087
	female	All comb				0.066
	male	All comb				
Total	both	Homogen.				0.091
	both	All comb		0.17	0.23	0.035

that an accumulation of speckled ANF in their relatives occurred on this basis.

Female relatives of SLE probands with marked arthritis symptoms had significantly higher frequency of ANFs in large joints. Initially the SLE probands who developed marked arthritic symptoms presented very high frequency of joint effusions (13). Speckled ANF was not related to joint effusions in female populations. The frequency of speckled ANF was increased in rheumatic male first degree relatives compared with non-rheumatic male first degree relatives (18) but the frequencies of ARA criteria of RA and of joint effusions did not differ in male relatives of SLE probands with and without marked arthritis.

The prevalence of homogeneous ANF was similar in relatives of SLE probands with and without marked arthritis, but combination of homogeneous nuclear staining and present or past history of joint effusions occurred more frequently in female relatives of SLE probands

with marked arthritis than in female relatives of probands without arthritis.

An aggregation of rheumatoid arthritis (RA) and of rheumatoid factors (RF) is also present in families of highly erosive RA, and RF is aggregated as continuous variable (15). Definite RA patients from hospitals and population surveys differ in frequency of RF and ANFs, which seem to be closely related in the latter type of population (9). However an accumulation of RA and RF does not occur in relatives or spouses of seropositive definite classical RA patients from hospitals (19). Two kinds of ANF are observed in the sera of RA patients: antibodies which give homogeneous nuclear fluorescence with nucleoprotein and liver nuclei, and antibodies which only react with liver nuclei giving speckled nuclear staining (11). Antinuclear factors are present in synovial fluids and sera of RA patients (1, 7) and granulocyte specific ANF are found to be associated with human RFs (6). The joint

deformities often differ in SLE and RA (10) In certain SLE patients a connection may exist between ability to produce speckled antinuclear factors and development of rheumatoid deformities in joints.

Antinuclear factors were related in SLE probands and their relatives, but the aggregation in relatives of SLE probands with high-titered ANFs was not particularly pronounced. However blood sample were taken for comparison of ANFs in probands with SLE whether or not the patients were in active stage of disease

Antinuclear factors (of speckled pattern and all patterns combined) occurred with higher frequency in first degree relatives of SLE probands with numerous LE cells at the time of SLE diagnosis than in relatives of probands with few LE cells at diagnosis. The frequency of ANFs in the corresponding populations of spouses did not, however differ significantly. The aggregation was not due to the size of sibships or families investigated (18)

These findings supplement previous observations indicating that certain lupus features are related to the presence of particular antibodies e.g., complement fixing antinuclear antibodies in lupus nephritis (9) An association between clinical features of SLE and antinuclear antibodies probably has to be sought at the time of SLE diagnosis. ANFs are obligatory in active SLE and the high prevalence of ANFs in relatives of SLE probands with marked arthritis or numerous LE cells at diagnosis may reflect a special power to produce a group or groups of antinuclear antibodies very extensively and these antibodies may somehow contribute to the phenomena used for selection of the probands.

The irregular appearance of ANFs in relatives of probands with SLE may be interpreted as evidence of polygenic inheritance (8) which is also assumed for allied disorders (16) The genetic mechanisms involved are probably very complex and remain to be elucidated. In family studies of SLE, selection of subgroups of probands with defined clinical and/or serological characteristics appears to facilitate this analysis.

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Early Diagnosis of Acute Myocardial Infarction with Special Reference to the Diagnosis of the Intermediate Coronary Syndrome

A clinical study

By Urbain Sawe

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ETIOLOGY AND DIAGNOSIS
OF ACUTE MYOCARDIAL INFARCTION
WITH SPECIAL REFERENCE
TO THE DIAGNOSIS OF THE INTERMEDIATE
CORONARY SYNDROME

— *A clinical study* —

by

URBAIN SAWE

STOCKHOLM 1972

Diagnose a matter of probability as those of
who follow the fat of our patient to post
mortem room know only too well

Sir George W. Pickering

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Previous History and Acute Symptoms in 921 Patients Admitted to a CCU

Acute central chest pain is the most prominent symptom in acute myocardial infarction. Yet pain with this location may be of other origins, and the interpretation of this symptom is an important problem in clinical medicine. Therefore with the introduction of specialized coronary care units (CCU) the interest of the symptomatology of acute myocardial infarction (AMI) has increased.

The diagnostic rate based on early appraisal is low today. Only half of the patients admitted subsequently fulfil the criteria for AMI (Lown et al. 1967, Wallace et al. 1967, Lundman et al. 1969, Amtrup et al. 1970, Askanas et al. 1970, Chiche et al. 1970, Engstedt et al. 1970, Hagbeldt, 1970, Asplund et al. 1971, Christensen et al. 1971, Armstrong et al. 1972, Engstedt et al. 1972).

The difficulties of early diagnosis consist of the following:

1. The border region from the still normal to the already pathological condition.

TABLE 1 The criteria for admission to the CCU of Serafimerlasarettet, Stockholm, Sweden

1. Central chest pain lasting for more than 15 min than the last 48 h.
2. Frank pulmonary oedema without previous known shlar lesion, urtema or intoxication.
3. Shock without suspicion of acute hypovolaemia or intoxication.
4. Syncope with electrocardiographic evidence of acute myocardial infarction.
5. Repeated bouts of chest pain with duration shorter than 15 min, status anginosus, within the last 48 hrs.

The last two criteria were added on September 18, 1968.

2. The non-characteristic onset of numerous pathological entities with equivocal symptoms (Frank 1970).

The CCU of Serafimerlasarettet, Stockholm, has used well defined criteria for admission (Table 1). The cardiovascular history for each patient has been recorded in specially constructed registration charts in numerical form (Lundman et al. 1968). These two circumstances have allowed a comparison of the history and the acute symptomatology for 1) patients, who were subsequently diagnosed to have an AMI and 2) patients, who were found not to fulfil the diagnostic criteria (OBS) (page 13).

The purpose of this part of the present study was to penetrate the following questions:

1. Are there any differences in the past history or the acute symptomatology between patients with subsequently verified AMI and the remaining patients admitted to CCU on the same criteria?
2. Can these differences be combined to build a diagnostic index?
3. How many true negatives can be excluded without missing any true positives with such an index?

MATERIAL AND METHODS

From January 1 1968 to January 1 1970 1099 patients were treated in the CCU of Serafimerlasarettet. The organization of the CCU general care, treatment of complication and after-care has been presented previously (Börck et al. 1969). Of these patients 450 (41 per cent) fulfilled the criteria for diagnosis of AMI. In 471 (43 per cent) this diagnosis could be excluded and in 125

TABLE 2 Admissions to the CCU in relation to the number of patients

	AMI		OBS	
	admissions	patients	admissions	patients
Admissions in both groups	88	69	99	69
More than one admission	50	23	80	34
One admission	31	312	92	292
Total	150	401	471	395

(11 per cent) the diagnosis was suspected acute myocardial infarction because of only partial fulfilling the criteria. The remaining 33 patients were admitted for other reasons: observation of arrhythmias or complications of catheterization and so on.

The present study has been limited to a comparison of the past history and symptomatology in the 450 patients with acute myocardial infarction (AMI patients) and in the 471 patients in whom this diagnosis could be excluded. These latter patients who were observed in the CCU will be called OBS-patients in the following.

Some patients were admitted more than once and as this study is based on admission to the CCU some patients are included in both diagnostic groups as is seen in Table 2. Altogether 69 patients were represented in both groups and 33 patients were admitted more than once in the AMI group and 34 patients more than once in the OBS-group. The remaining 312 AMI-patients and 92 OBS-patients were admitted once only. The 91 admissions therefore are made up of 730 individual

If one of the criteria for admission (page 11) is fulfilled the patient is transported to the CCU without delay. The same instruction is given to the staff of the general wards in respect of already hospitalized patients.

A junior and a senior physician are attached to the CCU during daytime and one physician is on duty at the unit during the rest of the week. About 20 different physicians specially interested in cardiology have thus been connected to the unit during the time of the study.

The history of the patients

At admission to the CCU all relevant data in the history of the patients were directly registered on specially constructed charts in numerical form (Lundman et al. 1968) for subsequent computer evaluation. After discharge the charts were checked against information from the hospital notes by the physicians attached to the unit. There are statements available on each patient about time of onset, delay, occurrence and type of pain, dyspnoea, clouding of the mind, unconsciousness, palpitation and vegetative symptoms at onset. Furthermore documentation includes previous angina pectoris and/or myocardial infarction. In all patients occurrence of previous hypertension, heart failure and diabetes mellitus is registered.

When patients gave uncertain information the code has been the negative for that parameter.

Electrocardiography

Routine ECGs including leads I, II, III, aVR, aVL, aVF, CR₁R, CR₁, CR₂, CR₄, CR₅, CR₇ were taken with an ink jet recorder on admission and every subsequent morning during the CCU stay.

Serum enzyme determinations

Blood samples for enzyme determination were taken on admission and on the following mornings for at least 3 days. The following enzymes were routinely determined: serum aspartate aminotransferase (S-GOT), serum alanine transferase (S-GPT), lactic dehydrogenase (LDH) and isoenzymes, LD₁ and LD₂, α -hydroxybutyrate

Adm p c d

Seraphimerlasarettet is a teaching hospital with 191 beds for general medicine. Acute admissions are received in the Casualty Department where a physician on duty makes preliminary appraisal.

dehydrogenase, (HBD) The analysis were performed in the Department of Clinical Chemistry at Serafinerlasarettet.

Criteria and definitions

Diagnostic criteria

The criteria for the diagnosis of AMI in the patients fulfilling the admission criteria to the CCU has been fulfilment of a, b or c.

(a) Appearance of a pathological Q wave and/or appearance or disappearance of a localized ST elevation followed by T inversion in at least two or more of 12 ECG-leads.

(b) Two S-GOT values of 40 U or more and with a maximum about 24 hrs after onset of symptoms in combination with lower S-GPT values with a maximum after about 36 hrs and/or two HBD values exceeding 75% of corresponding LDH values higher than 400 U with maximum about 60 hrs after the onset of symptoms, or a combination of one S-GOT—S-GOT value and one HBD-LDH value elevated as stated above.

(c) Findings at autopsy of myocardial necrosis of an age corresponding to the onset of symptoms.

Patients fulfilling the criteria for admission but not even partially the diagnostic criteria above have been classified as observation cases (OBS-patients).

Patients who only partly fulfilled the diagnostic criteria (e.g. one S-GOT value of 40 U/l or more, a localized ST elevation without T inversion etc) have been excluded from this study.

DEFINITIONS

Past history

Angina pectoris—Angina pectoris was defined according to the WHO criteria, substernal pain with or without radiation or left-sided precordial chest pain radiating into the left arm. For both alternatives onset on exertion and disappearance within 10 min. of the patient resting or taking nitroglycerine was required (Rose, 1962).

Previous myocardial infarction—The requirement for acceptance of patient report of a previous myocardial infarction diagnosed at a hospital.

Previous heart failure—A history of digitalis therapy or diuretics therapy given for symptoms of heart failure.

Hypertension—A history of high blood pressure treated with antihypertensive agents.

Diabetes mellitus—A history of diabetes mellitus treated with antidiabetic agents.

Statistics—Age has been calculated from 10 year class means.

Significance of differences between mean values were tested by Student's t-tests. The Chi-square test was used for testing the significance of differences of relative numbers. Yates correction was applied when small numbers were employed. Degrees of significance were tested at the 5% and 0.1 per cent levels.

RESULTS

Sex and age distribution

Sex

There were 284 men (63 per cent) and 166 women (37 per cent) in the AMI-group giving a male:female ratio 1.7:1. In the OBS-group there were 274 men (58 per cent) and 197 women (42 per cent) giving a sex ratio of 1.4:1. This difference is not significant.

Age

Fig. 1 shows graphically the age distribution in men and women in the study. The mean age was 66 years for the patients in the AMI group—63 years for men and 71 years for women. In the OBS-group of patients the mean age was 63 years—60 years for men and 67 years for women. The higher age for patients with AMI was significant for the entire patients groups ($p < 0.001$) as well as for men ($p < 0.01$) and women separately ($p < 0.001$). This is emphasised by the findings that 9 men (3 per cent) in the OBS-group were below 40 years of age but none in the AMI-group. Twelve women (6 per cent) in the OBS-group were below 50 years of age against none of the women in the AMI group as is shown in Fig. 1.

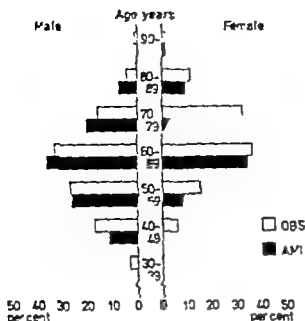


Fig. 1 Age distribution in relation to sex in the 450 AMI-patients and the 471 OBS-patients

Comments

The sex ratio i.e. males/females, is similar to previous Swedish studies from CCUs (Beverfjord *et al.* 1970, Ahlmark *et al.* 1971, Bostrom and Strum 1971, Brorson and Malmcrona 1971, Hennings and Holmberg 1971). There is a tendency to female overrepresentation in the OBS-group compared with the AMI-group but it does not reach statistical significance.

The mean age of the OBS-group was significantly lower for both men and women and this

might therefore provide a factor of discriminant importance. In accordance age below 50 is more common in the OBS-group and age above 70 is more common in the AMI patients.

PAST HISTORY

History of previous angina pectoris and myocardial infarction

There was no difference between the AMI and OBS-groups concerning a history of angina pectoris of either short or long duration as can be seen in Table 3. In the AMI group the incidence was 59 per cent—54 per cent for men and 67 per cent for women—against 56 per cent in the OBS-group—51 per cent for men and 62 per cent for women.

In the AMI group 33 per cent of the patients had a history of previous myocardial infarction against 39 per cent in the OBS-group; the difference again not reaching statistical significance.

Neither of these two manifestations of previous IHD was noted in 33 per cent of the patients in the AMI group and also in 33 per cent in the OBS-group.

CONCLUSIONS

The incidence of previous angina pectoris and myocardial infarction was similar in the two groups. Therefore previous angina pectoris and myocardial infarction are not discriminant factors between the two groups. It is of interest that a

TABLE 11 Incidence of previous angina pectoris (AP), myocardial infarction (MI) or none of these in IHD-manifestations in 450 AMI-patients and 471 OBS-patients admitted to CCL

		AMI			OBS		
		Males	Females	Total	Males	Females	Total
		n = 450	n = 450	n = 450	n = 471	n = 471	n = 471
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
None	AP	41	39	39	51	62	56
None	AP/MI	20	11	13	13	14	14
None	MI	33	34	33	42	36	39
None	AP/MI	29	33	33	5	30	33

None = no previous angina pectoris or myocardial infarction

TABLE 4 History of previous hypertension, heart failure and diabetes mellitus in 450 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males = 284 per cent	Females = 166 per cent	Total = 450 per cent	Males = 274 per cent	Females = 197 per cent	Total = 471 per cent
Hypertension	21	45	50**	11	51	17*
Heart failure	30	49	37*	19	57	35
Diabetes mellitus	7	16	10*	7	12	9*

N.S. ** $p < 0.001$

third of the patients in both groups have no history of previous angina pectoris and/or myocardial infarction.

The incidence of previous angina pectoris varies in different AMI-materials ranging from 22 per cent to 73 per cent (Rosenbaum-Levine 1941) and the incidence of previous myocardial infarction from 16 per cent to 31 per cent (Börck 1962 Bronson and Malmgren 1971). The percentage of patients without any of these two IHD-manifestations is generally not given in the literature.

Engstedt et al. (1970) showed that 34 per cent in their series of OBS-patients had had angina pectoris and 17 per cent gave a history of myocardial infarction. Hofvendahl (1971) has pointed out that there are two different categories of patients in the OBS-group: one with ischemic heart disease and one without and he also showed that none of the patients in the non IHD groups died within the first year after the discharge from the hospital, while the outlook for the first year is rather the same for all CCU patients with previous IHD whether an AMI is diagnosed or not.

History of hypertension, heart failure and diabetes mellitus

(as defined on page 13)

Table 4 shows the incidence of previous hypertension in the two groups of patients. Thirty per cent of the AMI-patients had a history of previous hypertension (21 per cent for men and 45 per cent for women). In the OBS-group the prevalence

was 17 per cent (6 per cent for men and 31 per cent for women). The difference between the two groups is highly significant ($p < 0.001$). A history of previous heart failure was given by 37 per cent of the AMI-patients against 35 per cent of the OBS-group. In this respect there was a difference between the sexes. Thirty per cent of the male AMI-patients reported previous heart failure against 19 per cent in the male OBS-patients which is significant. In the female patients there was no difference.

A history of diabetes mellitus was reported in 10 per cent of the AMI-patients—7 per cent for men and 16 per cent for women—and in 9 per cent of the OBS-patients—7 per cent for men and 12 per cent for women.

Comments

The incidence of previous hypertension varies in different studies and definitions differ (Chambers 1946—Henning and Holmberg 1971). With the present definition—a history of high blood pressure treated with antihypertensive agents—this factor was found to be a discriminating parameter. There is also a difference in this respect between the sexes within each group as well as when comparing men and women separately.

Heart failure in the past history was a not differentiating factor between the two groups, nor was diabetes mellitus which was reported by the same percentage in the two groups.

To sum up, history of previous treatment for hypertension is a discriminating factor between

TABLE 5 Time of onset of symptoms in 450 AMI patient and 471 OBS-patient

	AMI			OBS		
	Males n = 84 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 74 per cent	Females n = 197 per cent	Total n = 471 per cent
01-03	10	10	10	8	8	8
04-06	10	5	8	8	11	9
07-09	14	13	14	16	11	14
10-11	10	11	10	11	13	12
12-15	13	8	12	1	10	11
16-18	13	14	13	13	12	13
19-1	16	18	16	14	15	14
2-00	11	15	1	12	12	1
Uncertain	1	6	5	6	7	7

the two groups of patients while heart failure and diabetes mellitus in the past are represented roughly equally in the AMI and OBS-group.

Time of onset of symptoms and delay between onset and admission

In Table 5 the time during the day of onset of symptoms is shown. Onset of symptoms was more uncommon during night time 1 00-6 00 am but this finding was similar in both groups of patients.

The delay between onset and admission was about the same in the AMI and OBS-patients as is shown in Table 6. A delay of less than 6 hours was however a little more common in the AMI patients in comparison to the OBS-patients ($p < 0.01$).

Comments

Neither the time of onset of symptoms nor the time interval between the onset of symptoms and admission to CCU does separate the two diagnostic groups (Table 5 and 6). This delay mainly depended on the time taken for decision of seeking medical help by the patient.

In the present study those patients with a delay for more than 48 hours were not admitted. About 75 per cent of all patients regardless of final diagnosis were in fact hospitalized within 3 hours.

SYMPTOMS AT ONSET

As the criteria for admission to the CCU are stringent the incidence of atypical symptoms in

TABLE 6 Time between onset of symptoms and admission to CCU in 450 AMI patient and 471 OBS-patients

	AMI			OBS		
	Males n = 84 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 74 per cent	Females n = 197 per cent	Total n = 471 per cent
3 hours	44	1	41	39	39	39
6 hours	1	2	61	13	69	37
more than 6 hours					77	

TABLE 7 Chest pain before admission to CCU in 450 AMI patients and 471 OBS-patients

	AMI			OBS		
	Males = 284 per cent	Females = 166 per cent	Total = 450 per cent	Males = 274 per cent	Females = 197 per cent	Total = 471 per cent
Painless	6	7	6	8	8	8
Chest pain	27	16	23	31	31	32**
Chest pain with radiation	67	77	71	59	61	60*

* $p < 0.01$ ** $p < 0.001$

acute myocardial infarction cannot be estimated. In Table 7 the number of patients admitted to CCU without pain but fulfilling the admission criteria is shown. Six per cent of the AMI-patients were admitted because of fulfilling criteria 2-4 (page 11) and 8 per cent of the OBS-patients were admitted in the same way.

Chest pain

Table 7 shows the prevalence of patients with chest pain with or without radiation of pain in the two diagnostic groups. Pain without radiation occurred in 23 per cent of the AMI patients and in 32 per cent of the OBS-patients. Radiation of pain was reported by 71 per cent of the AMI patients and by 60 per cent of the OBS-patients. This latter difference is highly significant ($p < 0.001$).

Comments

The radiation of pain to the arms is highly significantly more common in the patients with

AMI compared to those admitted to CCU but found not to have AMI (OBS-patients) which has been shown in a prospective investigation (Sjwre, 1971). In the present study no further analysis of the distribution and radiation of pain could be done since in coding this parameter only occurrence of radiation has been noted. All patients, who have mentioned radiation from a central chest location to one or both arms, shoulders, neck and jaw back and to the epigastrium have been coded as having chest pain with radiation. A retrospective study of the details was therefore found impossible since the ordinary hospital records in most cases only give information of whether radiation of pain occurred or not. Still occurrence of radiation of pain was a discriminating factor between the AMI and OBS-patients.

Dyspnoea

Difficulty in breathing following the onset of symptoms was registered by 44 per cent of the AMI patients and 51 per cent of the OBS-patients.

TABLE 8 Dyspnoea before admission to CCU in 450 AMI patients and 471 OBS-patients

	AMI			OBS		
	Males = 284 per cent	Females = 166 per cent	Total = 450 per cent	Males = 274 per cent	Females = 197 per cent	Total = 471 per cent
No dyspnoea	38	51	36	55	41	49
Dyspnoea	51	35	52	36	44	39
Rattling respiration	11	14	12	9	15	12

TABLE 9 Disturbances in consciousness before admission to CCU in 430 AMI patients and 471 OBS patients

	AMI			OBS		
	Males n = 81 per cent	Females n = 166 per cent	Total n = 430 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
No disturbances of consciousness	83	76	80	79	74	77
Fainting	9	15	11	11	18	14
Unconsciousness	8	9	9	11	8	9

(Table 8) the difference not being significant. Twelve per cent in both diagnostic groups have noted rattling respiration.

Comments

About half of the patients mentioned that they were out of breath or felt shortness of breath previous to admission. At the registration of the description of this symptom complex, there was a separation of patients with dyspnoea and those with rattling respiration. These symptoms were similarly represented in the two groups of patients and therefore had no discriminating power

Disturbances in consciousness

Eleven per cent of the patients in the AMI group had felt some clouding of their mind following the onset of symptoms as had 14 per cent of the OBS-patients (Table 9). This difference is

not statistically significant. Unconsciousness was reported by 9 per cent of the patients in both groups.

Comments

In this study about 20 per cent of the patients regardless of the final diagnosis reported disturbances in consciousness in form of fainting or an unconsciousness, and a history of these symptoms does therefore not separate the two groups of patients. In the literature the incidence of these symptoms at onset of AMI varies from 3 per cent (Chambers 1946) to 28 per cent (Bean 1938).

Arrhythmic sensation

Palpitation was reported by 79 per cent of the AMI patients—21 per cent in men and 41 per cent in women—as can be seen in Table 10. In the OBS-group palpitation was registered in 40

TABLE 10 Arrhythmic sensation before admission registered and reported by 430 AMI patients and 471 OBS patients

	AMI			OBS		
	Males n = 81 per cent	Females n = 166 per cent	Total n = 430 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
Regularly	4	14	8	9	14	11
Irregularly	1	1	11	10	5	16
Fast heart			7	10	15	11
Slow heart			4	3	—	1
Arrhythmic sensation						
Total		41	79	4	52	41

$p < 0.001$

per cent—32 per cent in men and 52 per cent women. The difference between the two diagnostic groups was highly significant ($p < 0.001$) as was the difference in this respect between the sexes regardless of diagnosis. The sensation of palpitation has been divided into rapid regular and irregular action of the heart, of extra beats and of regular and irregular slow action of the heart. The most common complaint of arrhythmic sensation was irregular rapid action of heart.

Comments

There is a discrepancy in the present study between the subjective registration of palpitation in the patients and objective findings in the acute phase of myocardial infarction. In this study arrhythmic sensations were reported significantly more often in the OBS-patients and this parameter has therefore a discriminating power. This was a surprise bearing in mind the high incidence of different arrhythmias during the initial stage of AMI. Arrhythmic sensations were more common in women than in men regardless of the final diagnosis.

Autonomic symptoms

The findings are presented in Table 11. Nausea, or feeling sick, as an isolated autonomic manifestation was reported by 11 per cent of the AMI patients and by 19 per cent of the OBS-patients. This difference was highly statistically significant ($p < 0.001$).

Vomiting, sweating and combination of vegetative symptoms were much more common in the

AMI-patients when compared to the OBS-patients. These symptoms were present in 61 per cent of the AMI-patients and in 40 per cent of the OBS-patients ($p < 0.001$).

Comments

Nausea, or feeling a desire to vomit as an isolated symptom of autonomic disturbance was more common in the OBS-group than in the AMI group while vomiting, sweating and combination of these symptoms were more common in the AMI group.

Nausea is a vague symptom meaning that patient mentions that he is unwell and feels sick while the other vegetative symptoms are more distinct and based on central mechanisms (Wang and Borison, 1950; Borison and Wang, 1953).

To sum up nausea isolated is a discriminating factor speaking more for the OBS-diagnosis while vomiting, sweating and combination of autonomic symptoms strongly speaks for the AMI-diagnosis.

DISCUSSION

With the introduction of the CCUs the problem of an early diagnosis of AMI has become of great importance. The accuracy of an early diagnosis of AMI is, however, low. Many patients are admitted to the CCUs with a symptom complex compatible with an AMI yet without subsequent verification of the diagnosis.

Lown, 1967 has discussed the problem with the policy of admission to the CCU. He considered that the challenge of maximal prevention requires

TABLE 11 Autonomic symptoms before admission in 450 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males n = 284 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
No autonomic symptoms	29	26	28	41	53	41
Nausea feeling sick—only	10	13	11	18	21	19*
Vomiting, sweating and combination of these symptoms	61	61	61	38	44	40*

$p < 0.001$

TABLE 9 Disturbances in consciousness before admission to CCU in 430 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males n = 284 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
N disturbance of consciousness	85	76	80	79	74	77
Fainting	9	15	11	11	12	14
Unconsciousness	8	9	9	11	8	9

(Table 8) the difference not being significant. Twelve per cent in both diagnostic groups have noted rattling respiration.

Comments

About half of the patients mentioned that they were out of breath or felt shortness of breath previous to admission. At the registration of the description of this symptom complex, there was a separation of patients with dyspnoea and those with rattling respiration. These symptoms were similarly represented in the two groups of patients and therefore had no discriminating power.

Disturbances in consciousness

Eleven per cent of the patients in the AMI group had felt some clouding of their mind following the onset of symptoms as had 14 per cent of the OBS-patients (Table 9). This difference is

not statistically significant. Unconsciousness was reported by 9 per cent of the patients in both groups.

Comments

In this study about 20 per cent of the patients regardless of the final diagnosis reported disturbances in consciousness in form of fainting or unconsciousness, and a history of these symptoms does therefore not separate the two groups of patients. In the literature the incidence of these symptoms at onset of AMI varies from 3 per cent (Chambers 1946) to 28 per cent (Bean 1938).

Arrhythmic sensation

Palpitation was reported by 29 per cent of the AMI patients—21 per cent in men and 41 per cent in women—as can be seen in Table 10. In the OBS-group palpitation was registered in 40

TABLE 10 Arrhythmic sensations before admission registered and reported by 430 AMI-patients and 471 OBS-patients

	AMI			OBS		
	Males n = 284 per cent	Females n = 166 per cent	Total n = 450 per cent	Males n = 274 per cent	Females n = 197 per cent	Total n = 471 per cent
Rapid regular	4	14	8	9	14	11
Rapid irregular	10	12	11	10	23	16
Fast beats	6	7	6	10	15	11
Slow regular or irregular	1	6	4	5	—	1
Arrhythmic sensation of any kind	1	41	29*	32	52	40*†

p < 0.001

Computer Evaluation of Statistical Properties in the Early Differential Diagnosis of Patients Admitted to a CCU

The aim of part I was to study if there were any differences in the history of previous diseases and in the acute symptomatology between patients admitted to a CCU subsequently verified to have an AMI and those patients who did not fulfil the criteria of an AMI (the OBS-patients).

The aim of this part of the present study was to analyze the possibilities of working out a diagnostic index on basis of the results in part I.

The discriminative power of an index common to all patients has been evaluated through discriminative function analysis and its characteristics—sensitivity and specificity—are discussed.

MATERIAL AND METHODS

Parameters significantly discriminating between the 450 AMI patients and the 471 OBS-patients studied in part I are shown in Table 12. In seven parameters the chi-square value were more than 6.635 i.e. $p < 0.01$. Discriminant analysis was performed according to BMD 04 M (Dixon, 1965). The program used computes a linear function of number of variables measured on each individual.

This function can serve as an index for discrimination between two groups.

The statistical theory and method has been described by Kendall and Stuart (1966). Discriminant analysis with this method has previously been applied to medical data in association with AMI (Hughes et al. 1963, Lemlich 1965, Pipberger et al. 1968, Strubin et al. 1968, Cohn et al. 1972, Cotton et al. 1972 and Nyquist 1972).

The discrimination ability is expressed by a F value. The higher this F value is the better is the discrimination between the two groups.

Sensitivity i.e. the ability of a test to recognize the true positive and specificity i.e. the ability to find true negatives were calculated according to the following formulae (Cochrane and Holland 1971)

$$\begin{aligned}\text{Sensitivity} &= \frac{a}{a+c} \times 100 \\ \text{Specificity} &= \frac{d}{b+d} \times 100\end{aligned}$$

TABLE 12 Parameters significantly discriminating between 450 AMI-patients and 471 OBS patients

	AMI = 450 per cent	OBS = 471 per cent	P	χ^2
Age < 50 y	7	14	< 0.001	11.33
> 70 y	38	30	< 0.01	7.80
Delay < 6 h	61	57	< 0.001	3.99
Hypertension	30	17	< 0.001	22.00
Pain 1st radiation	71	60	< 0.001	12.58
Arrhythmic sensations	29	30	< 0.001	13.85
Nausea	11	19	< 0.001	12.69
Vomiting, sweating and combination	61	40	< 0.001	40.46

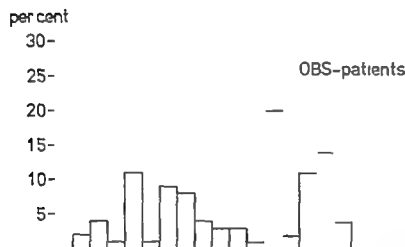
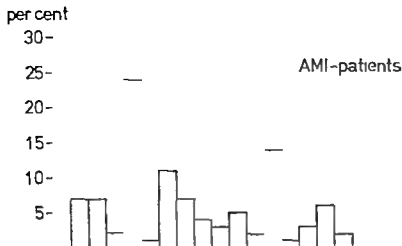


Fig 2 The discriminant scores of AMI- and OBS-patients, derived from the discriminant function analysis. The bars indicate the percentage of AMI and OBS-patients in each constellation of terms.

Radiation	+	+	-	+	-	+	-	+	-	+	-	+	-	-
Palpitation	-	+	-	-	+	+	-	-	+	+	-	-	+	+
Autonomic symptoms	+	+	+	+	+	+	+	-	+	-	-	-	-	-
Hypertension	+	+	+	-	+	-	-	+	-	+	+	-	-	-

a. individuals diagnosed as AMI and detected by the index (true positives)

b. individuals diagnosed as OBS but positive to the index (false positives)

c. individuals diagnosed as AMI but not detected by the index (false negatives)

d. individuals diagnosed as OBS and negative to the index (true negatives)

Discriminant analysis was performed on 300 consecutive patients in each diagnostic groups

(BMD 04 M) thereafter the procedure was repeated for all men and women separately and with exclusion of all patients without chest pain before admission.

RESULTS

Diagnostic index applicable to all patients admitted to CCU

This index was applied to all patients admitted to CCU

Radiation Palpitation				Radiation Palpitation			
↓	↓	↓	↓	↓	↓	↓	↓
14	3	2	6	20	12	4	14
24	11	3	7	11	9	3	8
7	7	1	2	2	4	1	1
4	5	1	2	4	3	2	1
Auton.sympt.				Auton.sympt.			
Hypertension				Hypertension			

AMI

OBS

Fig 3 Two modified Venn diagrams showing the intersection between the four parameters used in the discriminant function analyses.

The highest F value (14.0) was obtained for the combination of the following parameters: Radiation of pain, arrhythmic sensation, autonomic (vegetative) symptoms (not nausea isolated) and a history of hypertension.

Combinations with the following three parameters with chi-square levels above 6.635 ($p < 0.01$): Isolated nausea, age below 50 years and above 70 years were excluded because of lower F values.

AMI OBS-ratio

41 -

31 -

21 -

11 -

12 -

13 -

14 -



Fig 4 The ratio between AMI- and OBS-patients in each combination of the four parameters used in the discriminant function analyses.

Radiation	+	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Palpitation	-	+	-	-	+	+	-	-	+	+	-	-	+	+	-
Autonomic	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-
symptoms	+	+	+	-	+	-	-	+	-	+	+	-	+	-	-
Hypertension	+	+	+	-	+	-	-	+	-	+	+	-	+	-	-

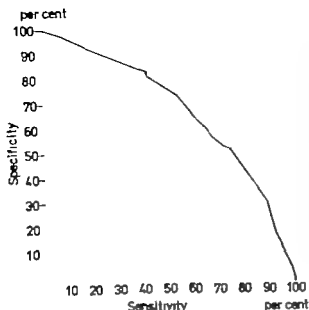


Fig 3 Specificity as function of sensitivity for the combination of the four items used in the test.

An attempt to find a more efficient separation of the two diagnostic groups through exclusion of all patients without chest pain before admission was not successful. Nor did discriminant function analysis for men and women separately improve index.

The calculated discriminant function coefficients

+0.00078 for radiation of pain

—0.00054 for palpitation

+0.00173 for combination of vegetative symptoms

+0.00092 for previous hypertension

The score (diagnostic index) for each patient was obtained by the following formula.

$$DI = 0.00078 R - 0.00054 P + 0.00173 V + 0.0009 H$$

where R = 0 if the patient is without radiation of pain and 1 if with radiation.

P = 0 if the patient has no palpitation and 1 if the patient has.

V = 0 if the patient has no vegetative symptoms and 1 if the patient has

H = 0 if the patient has no history of previous hypertension and 1 if the patient has.

The result of the analysis is presented in Fig. 2. The percentage of AMI and OBS-patients in each constellation of symptoms with decreasing score is presented in Fig. 2 and in Fig. 3 two modified Venn-diagrams (Feinstein 1969) show the intersection between the four parameters used in the two diagnostic groups of patients. The ratio between AMI and OBS-patients in each combination of items is shown in Fig. 4.

Specificity at different levels of sensitivity was calculated for the combination of items and is graphically illustrated in Fig. 5

When the sensitivity of the test was 89 per cent the specificity was 30 per cent meaning that exclusion of about a third of the OBS-patients would mean a loss of 11 per cent of the AMI patients.

Comments

The result of this analysis shows that there is a difference of the combination of symptoms in the patients admitted to our CCU but there is a big overlap in the constellation of symptoms. A low index speaks against a diagnosis of AMI but its ability to exclude this diagnosis is very moderate. The risk of making wrong decision in the judgment of the individual patient may however be minimized if the ratio between AMI and OBS-patients in each combination of items is taken in account.

DISCUSSION

There was no complete separation of the two diagnostic groups of patients. The previous history and the acute symptoms differ between patients with AMI and patients without but there is a considerable overlap. The discrimination could perhaps be better if not only symptoms were taken in account but also clinical signs at admission, e.g. respiratory rate, pulse pressure, systolic or diastolic blood pressure, heart rate and occurrence of rales. Another possibility of improvement of a diagnostic test would perhaps be if the results of some simple laboratory test at admission like the values of enzyme tests or an ECG were added.

Several authors (Lusted, 1965 and 1971 Sterling and Pollack, 1965 Bruce and Yarnall, 1966 Pipberger et al. 1968 Hall et al 1970 Bäck, 1972) have recently discussed in some detail fundamental problems which arise when medical diagnosis is subjected to rigorous mathematical treatment. The result of this discriminant function analysis ought to be interpreted mainly as an attempt to identify the symptoms which are essential for the description of AMI and more important for the differentiation between AMI and OBS-patients. Which are the specific question which need to be asked when we want to arrive at a medical diagnosis? How much and what type of information is required

The findings in this part of the present study led to an attempt to improve the enzymatic diagnosis by more frequent enzyme determinations and a more detailed analysis of the previous history and the acute symptoms and signs in a prospective series of patients in order to bring all these informations together and try to improve the diagnosing of AMI in its early hospital stay

SUMMARY

The discriminant power of an index common to all patients has been evaluated through discriminative function analysis and its characteristics—sensitivity and specificity—are discussed.

The best discrimination was obtained for the combination of the following parameters. Radiation of pain, arrhythmic sensations, autonomic symptoms and a history of previous hypertension.

The analysis shows that there is a difference of the combination of symptoms in the patients admitted to the CCU but there is a big overlap. A low index speaks against a diagnosis of AMI but the ability to exclude this diagnosis is moderate. Exclusion of about a third of the OBS-patients would mean a loss of 11 per cent of the AMI patients.

This finding prompted a prospective study with an improvement of the enzymatic diagnosis by more frequent enzyme determinations and a more detailed analysis of the previous history and the acute symptoms and signs in a consecutive series of patients admitted to CCU

Enzyme Diagnosis in Patients Admitted to a CCU with Special Reference to the Diagnosis of the Intermediate Coronary Syndrome

One of the reasons for a low diagnostic rate based on early appraisal could be an insufficient history-taking but another reason could be that some of the OBS-patients have a certain degree of myocardial damage not revealed by the ordinary routine of enzyme determinations as described in part I (page 12)

Since 1954, when LaDus and Wroblewski first measured the activity of serum glutamic oxaloacetic transaminase in myocardial infarction, this and other enzymes have been widely used as diagnostic aids.

S-GOT CPK and heat stable LDH are now the chief ones used clinically and it is wise also to order a S-GPT determination to avoid a misinterpretation—at the presence of hepatic involvement—of elevated S-GOT values (Hughes 1969, Åberg and Winfield 1972)

The purpose of the present study was to attempt to improve the enzymatic diagnosis of acute myocardial infarction. Parts of the result of this investigation is being published together with Bergsten (1972)

MATERIAL AND METHODS

The 191 patients described in part IV are the basis for this investigation. The criteria for admission to the CCU has been presented in part I (page 11)

Blood samples were drawn on admission and on four further occasions (just before the meals) within the next 4 hours. The blood was kept at 0°C and the serum separated within an hour. Centrifugation and storage was performed at -1°C. The blood samples were analyzed once

daily using a Reactions Rate Analyser (LKB 8600) connected to an evaluation unit (Optilab Bo Philip Instrumentation Stockholm). Reagents for S-GOT, S-GPT and LDH were from KABI AB Stockholm. Heat stable lactate dehydrogenase (LDH) was measured as LDH after preincubation of serum and LDH reagents except for α -ketoglutarate at 65°C for 30 min. CPH was determined with a kit (Monotest from Boehringer Mannheim, Western Germany)

Normal values for the enzymes used are presented in Table 13 (Bergström and Sjöwe, 1972)

DIAGNOSTIC CRITERIA BASED ON ENZYMES

The criteria for the diagnosis of AMI in this part of the present study have been the following: S-GOT values increasing to maximum above 40 U/l at more than one occasion 24–48 hours after onset of symptoms, simultaneously with or after an increase of CPK and following by a LDH curve with maximum later on and with a flat S-GPT curve. At S-GOT values with high S-GPT the enzymatic diagnosis was confirmed by the CPK and LDH curves only.

TABLE 13 Normal values for the enzyme used (Bergström and Sjöwe 1972)

Enzyme	Normal range	Borderline	Units
S-GOT	10—35	35—60	U/l (35°C)
S-GPT	10—35	35—60	U/l (35°C)
LD	100—550	550—400	U/l (35°C)
LD	50—30	30—300	U/l (35°C)
CPK	15—50	50—80	U/l (35°C)

TABLE 14 Diagnosis of Acute Myocardial Infarction by using frequent enzyme analyses and comparison with the ordinary routine

Intervals of analyses during the first 4 hrs	Acute Myocardial Infarction		Intermediate Coronary Syndrome and Suspected Infarction		Observation Cases	
	n	per cent	per cent		per cent	
Frequent (5/24 hrs)	88	46	19	10	84	44
Ordinary (2/24 hrs)	86	45	6	5	99	III

The criteria for the diagnosis of intermediate coronary syndrome (ICS) have been the following: the same type of S-GOT and LDH curves as seen in AMI but not exceeding the upper normal value (S-GOT = 40 U/l). The difference between the highest and lowest S-GOT value should be more than 10 U/l without changes in GPT and with a CPK curve typical for heart muscle damage. None of these patients had subsequently electrocardiographic evidence of typical AMI.

The criteria for the OBS-diagnosis have been the following: No fulfillment of the criteria for the AMI or ICS-diagnosis, i.e. normal constant enzyme values or other types of enzyme pattern.

RESULTS

AMI was diagnosed in 88 patients (46 per cent) and 19 patients (10 per cent) showed enzyme curves compatible with those found in AMI but with its maximum within the normal range giving

them the diagnosis of ICS. In 84 patients (44 per cent) the enzymes were III a constant level or with an atypical pattern resulting in an OBS-diagnosis.

The differences in diagnosis obtained with the use of frequent serum analyses during the first 24 hours compared to the ordinary routine described in part I is shown in Table 14. Two patients who would have had a diagnosis of suspected infarction with ordinary routine were diagnosed as having AMI and 15 patients who would have been diagnosed as OBS-patients with the ordinary routine were diagnosed as ICS.

Different types of enzyme pattern in AMI

Most of the patients in the AMI group had typical enzyme curves but in some of the patients the pathophysiological course was modified. The S-GOT-maxima in relation to S-GPT maxima in the present study are shown in Table 15 a. In Table 15 b the CPK-maxima are shown.

TABLE 15 a. S-GOT and S-GPT-maximum in the three diagnostic groups

	AMI = 88		ICS = 19		OBS = III	
	S-GOT	S-GPT	S-GOT	S-GPT	S-GOT	S-GPT
≤ 20		11	—	14	28	31
21—30	—	21	7	4	33	13
31—40	—	25	9	1	10	7
41—50	3	11	3	—	2	4
51—100	30	14	—	—	7	7
101—150	17	2	—	—	1	—
151—200	18	1	—	—	1	—
201—300	11	3	—	—	—	—
> 300		—	—	—	—	—

TABLE 15 b CPK-maximum in the three diagnostic groups

	AMI = 88	ICS = 19	OBS = 84
<50	1	3	46
50—80	1	6	17
81—150	17	9	11
151—300	29	—	4
301—600	22	—	—
601—1000	8	—	—
>1000	1	—	—
Not obtained	9	1	6

A few patients did not develop an enzyme curve at once indicating, in retrospect, a pre infarction period without enzyme leakage followed by a typical AMI-curve later on.

In some patients often with repeated retrosternal pains, status anginosus, before admission the enzyme curves have more than one maximum, indicating a prolonged course with gradual extension of myocardial necrosis.

The transaminase values were sometimes difficult to interpret. High S-GOT values combined with high S-GPT were seen in some patients possible supporting a hepatic involvement. In these patients the diagnosis was confirmed by a typical CPH-curve and LDH-curve or by electrocardiographic evidence of an AMI.

Five AMI patients died within the first 24 hours in the CCU and in these patients no S-GOT maximum was reached, but the start of the enzyme curves in these patients were typical and the diagnosis was verified at autopsy either macro- or microscopically.

Diagnosis of the Intermediate Coronary Syndrome

Nine men and 10 women fulfilled our admission criteria to the CCU showed a typical curve pattern of S-GOT values not exceeding the normal range and with its maximum during the first 24 hours in CCU. In 7 of these patients the difference between the highest and the lowest value of S-GOT

during the first 4 hours was 10—14 U/l and in the other 12 patients the difference was more than 15 U/l. The S-GOT and S-GPT-maxima for the ICS-patients are shown in Table 15 a and the CPK-maxima in Table 15 b. Two of the patients in this group showed ECG-changes as has been described by Prinzmetal et al. (1959) under the diagnosis of Variant Angina Pectoris. None of these 19 patients died during the stay in hospital.

Diagnosis of Observation case

Most of the patients in the group not fulfilling the diagnostic criteria for AMI or ICS showed constantly normal enzyme values, with a difference between the highest and lowest S-GOT value less than 10 U/l during the first 24 hours in CCU and in contrast to the ICS-patients these differences were not systematical. More than a fourth of the OBS-patients showed enzyme curves of different types mainly indicating a hepatic involvement with increased S-GOT and S-GPT values without a gradual increase to a defined maximum within the two first days at hospital. The enzyme maxima are shown in Tables 15 a and b.

DISCUSSION

Angina pectoris in the classical form described by Heberden and AMI in the equally classic picture of Herrick (1912) are the most well recognized clinical expressions of ischemic heart disease. Between these two, there is a condition that is more severe than angina and less severe than frank infarction, its significance has been uncertain and a source of controversy. This form of ischemic heart disease has been given a variety of names:

1. Preliminary pain (Fell, 1937)
2. Impending infarction (Sampson and Elaser 1937)
3. Coronary failure (Freedberg et al., 1948)
4. Intermediate coronary syndrome (Graybiel, 1951; Vakil, 1951)
5. Prodromal symptoms in myocardial infarction (Mounsey 1951)
6. Acute coronary insufficiency (Master et al 1956)

7. Premonitory pain (Maurice et al., 1956)
8. Preinfarction (Resnik, 1962 a, b)
9. Mikroinfarkt (Schneider 1968)
10. Infarctus rudimentaire (Delbecq 1969)
11. Acute coronary heart disease without recognizable infarction (Levine, 1971)

Intermediate coronary syndrome (ICS) is the term used throughout this study.

Vakul and Graybiel independently recommended this term in 1951. Vakul (1961) stated that ICS appears to be a fairly common and recognizable clinical entity usually self-limited, but with a propensity to develop AMI in the near future.

Gorlin (1972) stated that when the so-called intermediate syndromes between angina and infarction are suspected simply on the basis of severe chest pain and non-specific ST-T-changes, the likelihood of the diagnostic accuracy is reduced and remains in doubt without further investigation.

Jennings et al. (1957) showed that the S-GOT content of the infarction area of the myocardium after a ligation of the circumflex artery in dogs decreases soon after the 20—30 min of ischemia necessary to induce irreversible injury in most fibers of the posterior papillary muscle and indicates that there must be defects of sufficient size in the semipermeable membrane of the fibers to allow an enzyme molecule (S-GOT) with an estimated molecular weight of about 60 000 to leak out.

Rueggsegger et al. (1958) stated from their study of experimental myocardial infarction in dogs that reversible myocardial damage without necrosis, such as that seen in coronary insufficiency and pericarditis, does not appreciably influence the serum activity of S-GOT. According to these authors the serum concentrations of GPT often remain within normal limits following smaller myocardial infarcts in man.

In 1962 Resnik pointed out the possibility of very small myocardial necrosis which he called preinfarction and stated that there is evidence that in normal individuals, the maximal variation between the lowest and highest figures of S-GOT obtained over a period of days does not exceed

10 units. Hence figures of 10 and 36 in the same patient probably have as much significance as a maximal level of 45 in indicating that necrosis occurred according to Resnik.

Even Hughes (1969) considered more than ischemia to occur for S-GOT to be liberated from cardiac fibers and said that there must be necrosis with disintegration of cell walls.

Heindrich et al. (1970) however, pointed out that enzyme tests have not resolved the question of the clinical differentiation of angina pectoris as transitory myocardial ischemia from the more serious coronary attacks accompanied by myocardial necrosis. They showed a slight LDH-elevation in 20 per cent of their angina pectoris patients and stated that this indicated that occasionally the angina gives rise to a certain degree of myocardial damage but assumed that it would not be correct to call this condition by any other name than angina.

Krauss et al. (1972) have discussed these patients who fall between stable angina and acute infarction and defined prolonged coronary pain without enzymatic and electrocardiographic evidence of AMI as acute coronary insufficiency.

Several authors (Batzakis and Briere, 1967; Nozawa, 1970; Favoloro et al. 1971; Herman, 1971; Konttinen, 1971) have recently proposed a mild elevation of serum enzyme levels at the intermediate types of ischemic heart disease. Batzakis et al. (1967) said that in instances where modest rises are observed the source must be considered to be small and undetected areas of myocardial infarction and Herman (1971) stated that the underlying pathophysiology of the intermediate syndromes probably represents an acute change in the coronary circulation such as a subtotal occlusion of a vessel and that these conditions represent some degree of intramural or subendocardial tissue necrosis. Herman also pointed out the relationship between Prinzmetal's variant angina and intermediate coronary syndrome, supported by Poggi et al. (1971) who considered that "la crise d'angor spontané de Prinzmetal se rapproche plus de la phase aiguë de l'infarctus du myocarde que de l'angor spontané classique".

Fowler (1971) stated that preinfarction usually is defined by clinical criteria which are in part subjective. By definition no diagnostic alterations are shown in the serum enzymes. This was supported by Scanlon et al. (1971)

There is in fact a source of controversy on this subject (Friedberg, 1972) *In the present study there is a distinction between angina of unstable type which is defined as Fulton et al (1972) and intermediate coronary syndrome which is defined as an episode of chest pain causing the patient to seek medical help and accompanied by an increase of S-GOT values within the normal range with more than 10 units within 24—48 hours from onset of symptoms* In this diagnostic group there were 2 cases of Prinzmetal's angina supporting the hypothesis that this condition sometimes is not an angina but a small infarction.

The reason for having an intermediate diagnostic group is a still insufficient laboratory and clinical accuracy in differentiation between myocardial ischemia and myocardial necrosis.

According to Friedberg (1972) a major trend in diagnosis is to obliterate the sharp boundaries between no infarct, subendocardial infarct and transmural infarction and to regard myocardial infarction as a continuous spectrum in terms of severity and extent of necrosis from involvement of microscopic areas to fullblown transmural infarction.

Wilkinson (1971) said in his review over the future of diagnostic enzymology that the findings of diagnostic enzymology will always have to be interpreted in relation to the relevant clinical and other data, and Batsakis and Brere in their Interpretive Enzymology (1967) stressed that a positive result should be regarded as confirmatory and not diagnostic.

Blörck and Hansson (1956) showed the relation between S-GOT positive AMI and the clinical and electrocardiographic picture and pointed out that additional methods for the diagnostic decision in questionable cases would be of definite value.

In the present study most of the patients subsequently diagnosed as AMI patients had typical

enzyme curves. There were some patients with modified courses but in all these patients the frequent analyses with a combination of enzyme tests and ECG-changes were enough for establishing the diagnosis.

Several authors recommended a modified cardiac enzyme panel for investigation of chest pain (Hamolsky and Kaplan, 1961 Kibe and Nilsson, 1967 Coodley 1968 Rensch and Bunch 1968, Hughes, 1969 Albertini et al. 1970) There are great risks in judgement of single enzyme values. In the present study more than a fourth of the OBS-patients had atypical S-GOT-elevations.

Several reports are written about isotrogenic aberrations in this field (Batsakis et al 1967 Coodley 1968, Crowley 1968 Hughes et al. 1969 Popescu et al. 1969 Rowan, 1969 and Hammermeister and Merendino 1971 Sobel and Shell, 1972) The most common isotrogenic pseudo-myocardial syndrome is an opiate induced pressure increase in the biliary tract which may produce all of the symptoms and findings of myocardial infarction, in those in whom there is a biliary tract disease or in the absence of a gallbladder (Batsakis et al. 1967 Rowan, 1969) The S-GOT maxima in pseudo-myocardial infarct syndrome after administration of opiates are observed at five to eight hours after the administration (Batsakis et al. 1969) Isotrogenic elevation of CPK may occur with aqueous penicillin given intramuscularly four to five times the upper limits of normal (Rowan, 1969) Salkylates have been reported to elevate S-GOT (Hamolsky and Kaplan, 1961) Direct current counter shock therapy in the treatment of cardiac arrhythmias may produce elevated CPK and S-GOT values (Sobel and Shell, 1972)

Crowley (1968) pointed out that CPK is known to be elevated following vigorous muscular activity and following epileptic convulsions. Hemolysis produced by prosthetic valves may be associated with slight elevations of LDH (Hammermeister and Merendino, 1971)

There are several conditions which can confuse the enzymatic picture (Goldberg and Winfield, 1972, Sobel and Shell, 1972) The slow release of enzymatic material from the injured cellular

stocks at acute myocarditis does not cause characteristic changes (Popescu et al 1969)

Increased enzyme activity in cardiac failure and in pulmonary embolism has been reported (Sobel and Shell, 1972). A normal CPK confirms the absence of cardiac muscle participation as a cause of the rise of other enzyme activities and the LDH isoenzyme fractionation shows increased prominence of the hepatic fractions (Batzakis et al. 1967)

Acute pectoral myositis gave false positive CPK in noncoronary patients as did hypothyroidism, other active muscle diseases or cerebral hemorrhage.

The frequent serum analyses in the present study have given an increased accuracy in diagnosing the patients admitted to the CCU and revealed a group of patients with very small enzyme elevations and without typical ECG-changes

All the patients in this group have had previous ischemic heart diseases in their past history (page 35) supporting the final diagnosis of a coronary syndrome in these patients.

SUMMARY

Frequent serum enzyme determinations during the first 4 hours in the CCU have been done in 191 patients.

In 46 per cent the enzyme curves fulfilled the diagnostic criteria for acute myocardial infarction (AMI) and in 44 per cent this diagnosis could be excluded (OBS)

In the remaining 10 per cent the diagnosis was intermediate coronary syndrome (ICS) defined as an episode of chest pain causing the patient to seek medical help accompanied by an increase of S-GOT-values within the normal range but with more than 10 U/l within 24—48 hours from onset of symptoms and without typical ECG-changes suggestive of an AMI.

The accuracy of the diagnosis of ICS is discussed and a review over etiogenic aberrations and clinical conditions, which can confuse the enzymatic picture is made.

The Early Clinical Picture of Patients Admitted to a CCU

— a prospective study of 191 patients —

It is apparent from the study presented in part I that there are some differences between the patients admitted to the CCU on suspicion of having acute myocardial infarction whether this subsequently is verified or not.

Any convincing separation between the two diagnostic groups—AMI och OBS-patients—was not obtained with the discriminant function analyses presented in part II and based on the information in part I.

In an attempt to further improve the diagnostic accuracy in the early stage of acute myocardial infarction, a prospective study of consecutive patients admitted to CCU was performed.

The aims of the present study were:

- 1 to analyze all the patients systematically with a detailed questionnaire regarding previous history symptoms before onset of the attack leading to the hospitalisation as well as the symptoms of the acute attack (History and symptoms)
- 2 to collect similarly simple clinical parameters on admission to CCU like the blood pressure, the pulse pressure, the respiratory rate the heart rate and the occurrence of pulmonary rales (Signs)
- 3 to perform more frequent enzyme analyses than the traditional routine allowed (Tests) (Part III page 26)
- 4 and to bring all this information together in order to improve the diagnosis of AMI in its early stages.

MATERIAL AND METHODS

The series consisted of 36 consecutive admissions to the CCU Serafimerlasarettet from February 15 1971 to July 1 1971. These 236 admissions corresponded to 191 individual patients if

a patient has been treated in the CCU as an OBS-patient within one month before an admission because of an AMI he has been classified as an AMI patient and the previous episode has been judged as a prodromal symptom. A patient admitted to the CCU because of new symptoms within one month after an AMI without verification of a new myocardial infarction has also been classified as an AMI patient and the new admission interpreted as a status post myocardial infarction (Verghese and Lovell, 1966). Patients admitted more than once with the diagnosis of Observation have been classified as one OBS-patient, and the first episode has been the one included in this study.

The ordinary routine of the CCU

The organization and the ordinary routine of the CCU has been described in part I.

The history taking

The questionnaire

A questionnaire was constructed to cover systematically prodromal symptoms and acute symptoms and events leading to hospitalisation. Furthermore the history of previous diseases was included. The questionnaire was the basis for a personal interview by the author.

Interviews

During the day the patients were mostly interviewed in direct connection with the admission to the CCU. Patients admitted 19.00–07.00 were interviewed the morning following admission. In most of the cases neither the patient nor the interviewer had any information as to whether the symptoms were caused by an AMI or not. In some cases, however the interviewer who during the

study was on duty in the CCU knew the diagnosis because of ECG signs suggestive of AMI on admission. As far as was possible the patients were kept uninformed about this until the end of the interview. The interviews generally took about 20 minutes and usually the patients themselves gave all relevant information. In some cases a relative of the patient supplemented the information.

Addendum A retrospective study of the hospital records of all patients treated in our hospital during the same time with the diagnosis of AMI revealed 6 patients with AMI who have not been admitted to the CCU. Three men—one with a cerebrovascular accident, one with pulmonary oedema and one who was unconscious at the arrival to the Casualty Department and who died within one hour—and 3 women—all of advanced age with complex symptoms on admission. During the same time 2 other men were treated in the hospital with the diagnosis of suspected AMI because of small elevations of the enzymes.

Physical examination at admission

Systolic and diastolic blood pressure, pulse pressure, heart rate, respiratory rate and occurrence of rales or fulminating pulmonary oedema on admission to the CCU were noted in the chart of every patient. When the author was not the first physician to examine a patient this information was obtained from the ordinary registration charts described in part I.

Electrocardiography

The same method in recording the ECG described in part I was used (Page 12).

Serum enzyme determinations

The enzyme determinations used in this study are described in part III (Page 26).

DEFINITIONS

Angina pectoris, previous myocardial infarction, hypertension and heart failure are defined as described in part I (Page 13).

Diabetes mellitus A history of diabetes mellitus treated with antidiabetic agents.

Other diseases The patient's own information of previous diseases was accepted.

Statistics The same principles as in part I concerning significance of differences between mean values and of relative numbers (Page 13).

DIAGNOSTIC CRITERIA

Diagnosis based on electrocardiography

The same electrocardiographic criteria for the diagnosis of AMI outlined in part I were used (Page 13).

Diagnosis based on enzyme determinations

The enzymatic criteria of acute myocardial infarction are discussed and presented in part III. Enzyme diagnosis in symptoms leading the patient to a coronary care unit (Page 26).

RESULTS

Acute myocardial infarction (AMI) was diagnosed in 88 patients (46 per cent) according to the criteria in part III, and in 19 patients (10 per cent) the diagnosis was Intermediate Coronary Syndrome (ICS) also according to the enzyme result presented in part III. The remaining 84 patients (44 per cent) had no enzyme findings which could possibly be the basis for a diagnosis of AMI (OBS).

Sex and age distribution

Sex In the AMI group there were 66 men (75 per cent) and 22 women (25 per cent) giving a sex ratio of 3.1. In the ICS-group there were 9 men and 10 women giving a sex ratio of 1.1 which is similar to that in the OBS-group where there were 43 men and 41 women.

Age Fig. 8 shows graphically the age distribution for men and women in the present study. The mean age for AMI-patients was 65.6 years—64.1 for men and 70.4 for women. The ICS-patients had a mean age of 68.7 years—65.6 for men and 71.5 for women. The difference between

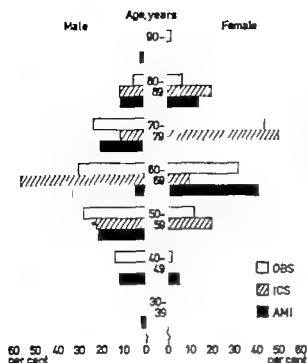


Fig. 6. Age distribution in relation to sex in the three diagnostic groups of patients.

the AMI and ICS-patients was not statistically significant. In the OBS-group the mean age was about the same as in the AMI group 63.7 years—62.1 for men and 69.3 for women.

Comments

In this study there were slightly more men than women in the AMI group compared to the corresponding group of patients described in part I. The mean age of the men in all three diagnostic

groups was lower than the mean age of the corresponding women. It was higher in the ICS-group than in the two other groups. One man in the AMI group was below 40 years of age and this man was from Turkey. One Swedish woman in the AMI group was below 50 years of age. The AMI-diagnosis in men below 40 and women below 50 years of age is extremely uncommon in Sweden (Björck 1960 a, Wiklund 1971, Ahlmark and Gillgren 1972). The age distribution corresponds with that presented in part I.

A PAST HISTORY

First there will be presented an analysis of the occurrence of diseases traditionally associated with AMI as angina pectoris, previous myocardial infarction, hypertension, heart failure and diabetes mellitus. Other diseases in the past history are described under the heading of Co-morbidity.

Previous angina pectoris

Angina pectoris with a duration of less than 1 month prior to admission has been classified as prodromal symptom and not counted here. A history of angina pectoris was given by 47 per cent of the AMI patients and by 58 per cent of the OBS-patients. Only 1 patient in the ICS-group was without history of angina pectoris (Table 16).

Angina pectoris with a duration of less than 6 months prior to admission occurred in 9 per cent of the AMI patients against 1 per cent of the OBS-patients while none of the patients in the

TABLE 16. The incidence and duration of previous angina pectoris in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 2 per cent	Total = 68 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females n = 41 per cent	Total = 84 per cent
N. angina	4	33	33	11	—	3	11	39	42
< 6 m	11	3	9	—	—	—	—	—	1
6 m—2 yrs	6	3	6	11	—	3	3	3	3
2 yrs—10 yrs	13	3	1	67	40	53	28	7	27
> 10 yrs	15	11	15	11	60	57	21	79	25

ICS-group mentioned angina pectoris of short duration. The difference between AMI and ICS-patients was highly significant and significant between the OBS- and ICS-patients.

Six per cent of the AMI patients reported on angina pectoris with a duration of more than 6 months but less than 2 years. Angina pectoris of this duration was indicated by 5 per cent in each of the other two groups of patients.

Angina pectoris with a duration of more than ten years occurred in 15 per cent of the AMI patients against 25 per cent of the OBS-patients while more than a third of the ICS-patients had a history of angina pectoris lasting for more than 10 years. The difference between AMI and ICS-patients was almost significant ($p < 0.05$).

Thirty eight per cent of the AMI patients used nitroglycerine against 56 per cent of the OBS-patients. Nearly all the patients in the ICS-group used nitroglycerine (90 per cent).

Comments

The incidence of angina pectoris was about the same in the AMI and OBS-groups, while it was considerably higher in the ICS-group.

The incidence of angina pectoris in patients with AMI is reported to be about 45–50 per cent in several Swedish investigations on AMI (Björck, 1962, Bevegård et al. 1970, Bronson and Malmcrone, 1971, Henning and Holmberg, 1971). In studies from other countries the incidence varies from 40–65 per cent in different AMI-materials. (Billings et al. 1949, Chambers, 1946, Moss, 1964, Lown, 1968 and Bailey et al. 1968).

The occurrence of angina pectoris in patients admitted to the CCU without a subsequent verification of AMI is difficult to estimate from the literature. Engstedt et al. (1970) noted 34 per cent in their OBS-group and in Hofvendahl's series (1970) 50 per cent gave a history of angina pectoris and/or myocardial infarction.

The problems with unstable angina pectoris, prodromal angina and pre-infarction-angina are discussed under the heading of Prodromes (page 39).

Previous myocardial infarction

Table 17 shows the incidence of previous myocardial infarction in the three diagnostic groups. Thirty two patients (36 per cent) in the AMI group gave a history of previous myocardial infarction and 32 patients (38 per cent) in the OBS-group while 11 (38 per cent) of the 19 ICS-patients had a previous myocardial infarction in their past history. None of these differences were significant.

Comments

With the introduction of the CCUs the hospital mortality of acute myocardial infarction has been reduced (Hofvendahl 1971 and Christiansen et al. 1971). This fact ought to lead to an increase of the prevalence of previous myocardial infarction in patients admitted to the CCU. In this study the incidence is rather high but corresponds to the findings in part I. In other Swedish studies the incidence varies from 17 per cent to 31 per cent.

TABLE 17 The incidence of previous angina pectoris (AP) and myocardial infarction (MI) in the three diagnostic groups and the incidence of no previous IHD-manifestation in the same groups of patients

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Previous AP	47	47	47	89	100	95	56	61	58
Previous MI	36	36	36	44	70	58	44	32	38
N previous IHD	41	46	42	0	0	0	40	34	37

(Bevegård et al 1970 Henning and Holmberg, 1971 and Brorson and Malmcrona 1971) One reason of the high incidence of previous myocardial infarction is perhaps that there is no upper age limit in our admission criteria.

No previous IHD manifestations

Previous IHD-manifestations were defined as occurrence of angina pectoris and/or myocardial infarction in the past. Thirty seven patients (42 per cent) in the AMI-group were without history of previous IHD and 31 (37 per cent) in the OBS-group while none of the ICS-patients were without IHD (Table 17)

Comments

The number of patients without previous manifestations of IHD in the AMI group is in accordance to the findings in part I (page 14) and to other reports (Brück 1962 Lönn 1967 Wiklund 1971) The similarity in this respect between the AMI and OBS-patients is interesting.

Remarkably all the patients in the ICS-group had a history of angina pectoris and/or myocardial infarction. Only 31 patients (16 per cent) were without manifestations of IHD at the time of this investigation but the immediate constellation of symptoms in these patients does not exclude the onset of IHD

Hypertension, heart failure and diabetes mellitus

The incidence of hypertension was 19 per cent in the AMI group, 21 per cent in the ICS-group and 18 per cent in the OBS-group (Table 18)

Previous heart failure was reported by 36 per cent of the AMI patients, 68 per cent of the ICS-patients and 54 per cent of the OBS-patients (Table 18) The differences between the AMI patients and the two other groups were almost significant ($p < 0.05$)

In Table 18 it is shown that 7 per cent of the AMI patients had diabetes mellitus before admission—3 per cent of the men and 18 per cent of the women. In the ICS-group 4 patients had diabetes mellitus—1 man and 3 women. The incidence of diabetes in the OBS-group was 5 per cent—2 per cent in the men and 7 per cent in the women

Comments

The incidence of hypertension ranges from 14 to 74 per cent in the literature (Thomas et al 1968 and Chambers, 1946) Some authors have pointed out that hypertension is more common in women (Billings et al 1949 Maurice et al 1954) In this study there was no statistical difference between the sexes and no differences between the three diagnostic groups.

The incidence of previous heart failure was about the same in the AMI patients as in the corresponding patients in part I In this study there was an almost significant difference between the AMI patients and the two other groups. The incidence of heart failure was high in the ICS-group

In the literature the incidence of heart failure in the past history of patients with acute myocardial infarctions differs from 11 per cent (Chambers, 1946) to 42 per cent (Brorson et al 1971)

TABLE 18 The incidence of previous hypertension, heart failure and diabetes mellitus in the three diagnostic groups

	AMI			ICS			OBS		
	Males n 66	Females n 2	Total n 68	Males n 9	Females n 10	Total n 19	Males n 43	Females n 41	Total n 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Hypertension	15	5	19	22	20	21	7	29	18
Heart failure	30	55	36	11	70	68	17	61	51
Diabetes	5	18		11	30	21	2	7	5

Concerning diabetes mellitus the incidence of this disease was low in the men in the AMI group while the incidence in the women corresponds to that found in the literature.

Diabetes mellitus occurred in 8–17 per cent in different series of AMI patients (Chamber 1946, Billings et al., 1949 Plotz, 1957 Wahlberg, 1963 and Sievers, 1964) The incidence is commonly higher in women than in men (Sievers, 1964, Bailey and Beaven, 1968) which was confirmed in this study as well as in part I

The occurrence of diabetes mellitus was high in the group of patients with slight elevations of the enzyme values after the onset of symptoms (The ICS-group)

Co morbidity

Any review over the co-morbidity in patients with IHD has not been found in the literature. The occurrence of different diseases in the patients of this study is presented Table 19

Peptic ulcer

Eighteen AMI patients (21 per cent) gave history of this disease. The same incidence was indicated in the ICS-group and in the OBS-group. 13 patients (16 per cent) gave a positive answer in this respect.

The incidence of this condition was higher in

the men in the AMI and ICS-group than in the corresponding women ($p < 0.05$) but about the same in both sexes in the OBS-group (Table 19)

Renal calculus

The incidence of a renal calculus was high in all diagnostic groups (10–16 per cent) However there were no differences between the groups.

Gout

Only a few of the patients gave a history of gout. One patient in the AMI-group, 4 in the OBS-group and none in the ICS-group gave a history of this disease.

Diseases of the respiratory system

Asthma bronchiale, chronic bronchitis, emphysema or healed pulmonary tuberculosis have been classified as chronic respiratory diseases of importance in the past history. History of acute pneumonia, common cold and other more temporary diseases has not been evaluated in this study

A history of chronic respiratory diseases was positive in 11 AMI patients (13 per cent) in 1 ICS-patient (5 per cent) and in 7 OBS-patients (8 per cent)

Diseases of the urinary tract

Pyelonephritis or cystopyelitis occurred in 4 AMI patients and in 5 OBS-patients.

TABLE 19. The co morbidity in the three diagnostic groups of patients

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Peptic ulcer	24	9	21	44	—	21	16	15	16
Renal calculus	18	5	14	22	10	16	9	10	10
Gout	2	—	1	—	—	—	7	—	5
Resp. disease	15	5	15	—	10	5	2	15	8
Pyelonephritis	6	—	5	—	—	—	9	2	6
Hepatic and biliary disease	14	46	22	—	30	16	12	2	17
Gout	5	9	5	—	—	—	5	2	4
Mal. pregnancy	—	5	1	—	—	—	—	2	4
Cerebrovascular disease	2	9	5	—	—	—	2	7	5
Other	5	9	6	—	10	5	9	12	11

Hepatic and biliary disease

In 19 AMI patients (22 per cent) in 3 ICS-patients (16 per cent) and 14 OBS-patients (17 per cent) there was a positive history of disease of the liver or the gall bladder mainly in form of cholecystitis with or without stones. The incidence was higher in women in the AMI and ICS-group than in the corresponding men ($p < 0.001$) but about the same in both sexes in the OBS-group.

Gout

Four AMI patients and 3 OBS-patients gave a positive history of diseases in the thyroid gland.

Malignancy

One AMI patient had been operated because of a neoplasm of the rectum, while four OBS-patient had a history of malignancy: myelomatosis, reticulocell sarcoma, neoplasm of the sigmoidum and in the urinary bladder.

Cerebral lesions

Four AMI patients and four OBS-patients had had cerebrovascular accidents with different degrees of remaining symptoms.

Comments

The incidence of peptic ulcer in patients with coronary disease varies in the literature. Sievers (1964) showed that 71 patients in his study had had peptic ulcer—9 per cent in men above 60 years and 6 per cent below 60 and 3 respectively 1 per cent in the corresponding ages in the women. Watkinson (1956) who based his conclusions on autopsies, said that there was a significant relation between coronary sclerosis and ulcers in men, especially in younger ages.

In the present series the incidence was rather high. One explanation may be that a systematical questioning of the patients gives a higher incidence than retrospective studies of hospital records. The incidence of these complaints was higher for men than for women in the AMI and ICS-group.

Asl. Upmark and Adner (1950) pointed to an association between gout and coronary heart disease. The result of the study does not confirm

this observation, but the present series may be too small in this respect.

Thomas et al. (1968) found chronic bronchitis in 10 per cent of their AMI patients.

Concerning bile stone, Johansson (1959) has given a incidence of 2 per cent and Billings et al. (1954) 13 per cent. Forrester et al. (1970) showed that the incidence of gall bladder disease is not greater in patients with than without coronary heart disease.

In the present study the incidence was higher in the women in the AMI and ICS-group compared to the men in these groups, while the incidence was about the same in both sexes in the OBS-group.

Other diseases analyzed in the present study were represented in about the same incidences in all three diagnostic groups.

To sum up the co-morbidity the following can be concluded: Peptic ulcer, renal calculus and gall stone were common previous conditions in the patients admitted to the CCU. There were no particular differences between patients subsequently diagnosed as AMI patients, ICS-patients or OBS-patients. Other diseases were more uncommon and with unsystematic occurrence in the three diagnostic groups of patients.

Smoking Habits

The smoking habits of the patients in this study are shown in Table 20. About half of the OBS-patients and half of the ICS-patients had never smoked against only 19 per cent of the AMI patients ($p < 0.001$).

A fourth to a third of the patients in all three diagnostic groups were former smokers.

Fourty per cent of the AMI patients were cigarette smokers against 11 per cent of the ICS-patients and 23 per cent of the OBS-patients.

Cigar and pipe smokers were represented in 14 per cent of the AMI group, 11 per cent in the ICS-group and 4 per cent in the OBS-group.

As can be seen in Table 20 the smoking habits varied between the sexes but the differences between the diagnostic groups are still significant if the sexes are studied separately.

Comments

The incidence of a positive history of smoking was higher in the AMI group than in the two other groups.

This difference is interesting as nearly all patients in the present study have previous IHD manifestations and the smoking habits would be expected to be nearly the same in the three different groups.

Gorbatow (1961) noted that 71 per cent of the AMI patients were smokers against 55 per cent in a control group. He also pointed to the different smoking habits in men and women. Engstedt et al. (1971) showed an incidence of 40 per cent in the AMI patients and 41 per cent of their OBS-patients. Henning and Holmberg (1971) found 70 per cent in their study of AMI-patients.

To sum up, the incidence of smokers or former smokers was higher in the AMI group than in the two other groups. This in spite of a high incidence of IHD manifestations even in these two latter groups.

B PRODROMES

Increased knowledge about the course of the AMI with awareness of the high initial mortality has caused many authors to focus interest on the time before the onset of the acute event in order to discover prodromal symptoms.

Solomon et al. (1969) maintained that 65 per cent of the patients in their study had prodromes

of some kind. Renggli (1971) related an incidence of 70 per cent and Hochberg (1971) not less than 84 per cent.

In the older literature the incidence of prodromes varies greatly and Mounsey (1951) who in his series indicated an incidence of 29 per cent, pointed out that the discrepancy between different studies could depend on different compositions of the materials and on different opinions about what constitutes prodromes.

The importance of continuing studies of this subject has recently been emphasized by a WHO Working Group on the Prodromal Symptoms of Myocardial Infarction and Sudden death (1971).

The time defined as prodromal time differs. Solomon (1969) considered two months as reasonable and Mounsey (1951) three months. Renggli (1971) in her study estimated one month as the period of prodromes.

In the present study prodromes have been defined as symptoms started or aggravated within one month before admission to the CCU.

Methods

The 191 patients in the present study were systematically asked about occurrence of new or increasing symptoms appearing during the last month.

By asking the patient if the acute attack has started like a bolt from the blue, the patient usually understood the purpose of the question.

TABLE 70 Smoking habits in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Non smokers	12	41	19**	11	80	46	23	75	47**
Former smokers	3	14	17	56	10	32	33	13	24
Cigarette smokers	58	45	40	11	10	11	33	10	23
more than 30-day	3	5	3	—	—	—	3	—	2
Pipe-Ciga smokers	18	—	14	2	—	11	7	—	4

p < 0.5 ** p < 0.001

The patient was asked if he during the last month before admission had noted any new or uncommon symptoms of some kind. After that he was systematically asked about the following symptoms in layman terms

- Onset of angina pectoris
- Crescending angina pectoris
- Recent onset of atypical pains in chest or arms
- Onset or worsening of dyspnoea
- Onset of dizziness
- Onset of progressive or new weakness or fatigue
- Onset of new symptoms from the gastrointestinal organs in form of flatulence, obstipation or diarrhoea
- Loss of appetite
- Onset of profuse sweating
- Onset of arrhythmic sensations

The prodromal time was noted for each patient.

The patients were also asked if they had visited a physician during the last 3 months either for a regular check up or on account of new symptoms.

Results

There was some change of the symptomatology in 68 per cent of the AMI patients within one month before admission to CCU (Table 21). The

patients in the ICS-group reported prodromes in 53 per cent while 46 per cent of the OBS-patients had noticed onset or changing of symptoms during the month before the attack leading the patient to the hospital. The difference between AMI and OBS-patient was highly significant ($p < 0.001$)

Pain as a prodrome

The most common prodrome before admission was new or increasing chest pain. This was reported by 55 per cent of the AMI patients 47 per cent of ICS-patients and 24 per cent of the OBS-patients. The difference between the AMI and the OBS-patients was highly significant ($p < 0.001$)

Atypical pain as a prodrome

Onset of atypical pain in chest or arms was reported by 16 per cent of the AMI-patients. Some patients described a discomfort in the chest in the form of a pricking sensation, a traction in the right side, a stitch or a spasm. Some patients had had an onset of pain in the right or left arm. These uncharacteristic descriptions occurred also in 11 per cent of the ICS-patients and in 16 per cent of the OBS-patients.

TABLE 21 Prodromata. A comparison of new or crescending symptoms one month before admission to CCU between the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 42 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total n = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 81 per cent
Prodromes of any kind	71	59	68	56	50	53	42	51	46
Chest pain	56	50	55	56	40	47	26	22	24
Dyspnoea	4	18	23	—	—	—	21	27	1
Tiredness	77	36	50	11	70	16	12	44	7
Autonomic symptoms	15	5	13	—	20	11	9	17	13
Loss of appetite	11	9	10	11	10	11	12	20	16
Arrhythmic sensations	6	23	10	2	—	11	16	15	16
Flatulence, diarrhoea	11	27	15	11	10	11	23	10	17
Vertigo, dizziness	6	—	5	11	30	1	8	79	19*
Headache	3	—	—	—	10	5	5	5	5
Miscellaneous descriptions	1	14	16	—	20	11	14	17	16

$p < 0.01$ $p < 0.001$

Dyspnoea as a prodrome

Appearance or worsening of difficulties in breathing was reported by 23 per cent of the AMI-patients and 24 per cent of the OBS-patients while none of the patients in the ICS-group had noticed any changes in this respect.

Weakness or fatigue as a prodrome

Onset of an unnatural weakness or fatigue was mentioned by 30 per cent of the AMI-patients 16 per cent of the ICS-patients and 27 per cent of the OBS-patients.

Other symptoms as prodromes

Onset or worsening of arrhythmic sensations during the month before admission was found in 10 per cent of the AMI-patients and in 11 per cent of the ICS-patients while 16 per cent of the OBS-patients had noticed this.

As can be seen in Table 21 the incidence of other symptoms varies and was more uncommon. Onset of vertigo, dizziness, sensation of light headness was indicated by 5 per cent of the AMI-patients while 21 per cent of the ICS-patients and 19 per cent of the OBS-patients had experienced of this kind.

Loss of appetite was reported by 10 per cent of the AMI-patients, 11 per cent of the ICS-patients and 16 per cent of the OBS-patients.

Autonomic symptoms, usually sweating, had started in 13 per cent of the AMI-patients, in 11 per cent of the ICS-patients and in 13 per cent of the OBS-patients.

Two per cent of the AMI-patients had got

headache of a kind they previously not experienced the last month before admission, so had 5 per cent of the ICS-patients and 5 per cent of the OBS-patients.

Disturbance of gastrointestinal functions in form of onset of flatulence diarrhoea or constipation was reported by 15 per cent of the AMI-patients, 11 per cent of the ICS-patients and 17 per cent of the OBS-patients.

The prodromal time

In 7 per cent of the AMI-patients the prodromes began the day before admission. The symptoms thus defined as prodromes were not the same immediate symptoms leading the patients to the hospital. Eleven per cent of the ICS-patients and 4 per cent of the OBS-patients also reported prodromes the day before onset of the immediate attack.

Prodromes with onset within one week before admission were found in 30 per cent of the AMI-patients, in 16 per cent of the ICS-patients and in 23 per cent of the OBS-patients.

A prodromal time of a fortnight was reported by 12 per cent of the AMI-patients, 5 per cent of the ICS-patients and 7 per cent of the OBS-patients.

Prodromes with a duration of more than fourteen days, but starting within a month before admission occurred in 26 per cent of the patients in the AMI-group and in 32 per cent of the ICS-group while 17 per cent of the OBS-group mentioned prodromes of that length. (Table 22)

TABLE 22 Prodromal time in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
N prodromes	29	11	32	41	50	47	18	51	54
1 month	29	18	26	33	50	32	9	24	17
2 weeks	15	5	12	11	—	5	2	12	7
1 week	20	32	25	11	—	5	25	12	19
1 day	8	5	7	—	20	11	5	2	4

Visiting a physician before admission

To clarify how the patients act on prodromes they were asked if they had visited a physician during the last months before admission, either because of a routine check up or because of new symptoms.

Regular contact with a physician because of chronic disease was reported by 25 per cent of the AMI patients, by 32 per cent of the ICS-patients and by 38 per cent of the OBS-patients.

A further 34 per cent of the AMI patients had seen a physician within the last month before admission because of onset of new symptoms and in half of these cases they had seen the physician within the last week. In the ICS-group the figures were about the same—32 per cent and 16 per cent. Twenty nine per cent of the OBS-patients had also seen a physician the month before admission because of new symptoms and a third of these in the last week.

Thirty two per cent of the AMI patients were without any contact with a physician the last three months before admission which was also the fact in 25 per cent of the ICS-patients and in 2 per cent of the OBS-patients. (Table 23)

Discussion

There are considerable difficulties inherent into an evaluation of the occurrence of prodromes in patients admitted to hospital with an acute attack of chest pain. Retrospective studies can give false interpretations of atypical symptoms. Handsight may affect the patient's description of his symptoms as well as the doctor's interpretation of them (Fulton et al. 1972)

A patient knowing himself as being in the initial phase of an AMI may overemphasize symptoms like tiredness and even progressive chest pain before onset of the immediate attack. Furthermore, studies do not reveal how often such symptoms do not end with an AMI.

In this study the patients were interviewed very early after the onset of symptoms which had brought them to hospital. Generally the patients were not aware if the symptoms had been caused by an AMI or by something else. However all patients knew of the suspicion of an AMI as they had been admitted to a CCU and thus hindsight ought to play the same role in all these diagnostic groups.

The incidence of prodromes of some kind was high in all three diagnostic groups but the difference between the AMI and OBS-patients was highly significant. This difference was mainly caused by the development of a new or increasing chest pain. Other symptoms were of less discriminative importance as the incidence was about the same in the three groups. Dyspnoea, fatigue, atypical pain, sweating and loss of appetite occurred equally often in the three groups. Many of these symptoms are common in the general population and may precede other disease processes.

The number of patients visiting a physician within a month before admission was high in all three groups. This is in accordance with several authors (Kuller et al. 1966 Stowers et al. 1970 Wiklund 1971 Fulton et al. 1972)

One third of the AMI patients had visited a doctor during the preceding month but the same proportion of the OBS-patients had also done so.

TABLE 3 The incidence of contact with a physician before admission to CCU in the three diagnostic groups

	AMI			ICS			OBS		
	Males n = 64	Females —	Total n = 79	Males n = 9	Females n = 10	Total n = 19	Males n = 43	Females n = 41	Total n = 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Routine check up			5	4	3	3	4	31	38
After 1 the last 3 months				1	1	11	1	10	11
After 1 the last month		1		11	2	16	14	4	19
Within the last week						16		12	10

This reveals that the early judgement of these patients is very difficult. Also about a third of the ICS-patients had seen a doctor within the last month. There were several reasons for seeing a doctor: for instance atypical chest pain, common cold, abdominal pain and routine check up. Only a 25 to 30 per cent of all the patients were without medical control the time before admission.

Mounsey (1951) indicated premonitory symptoms in 29 per cent of his AMI patients. Maurice et al. (1954) reported 40 per cent and Steifa and colleagues (1962) 45 per cent in their study. In other studies (Solomon et al. 1969, Moss 1970, Stowers et al. 1970, Renggl 1971, Skjaeggstad 1971, Furberg et al. 1972) the incidence varies from 50 to 70 per cent. Only a few authors have given information about prodromes in patients without AMI. In Solomon's investigation (1969) none of the 25 control patients gave a history of prodromes. Ander et al. (1971) found chest pain before admission in 76 per cent of the cases with AMI and only in 12 per cent in a screening group of patients. Hochberg (1971) pointed out that the occurrence of prodromes was high even in a group of patients with what he called acute coronary insufficiency without AMI. His figures were 93 per cent in a group of patients with acute coronary insufficiency compared with 84 per cent in an AMI group.

Prodromes occurred in several patients in the present study who did not develop myocardial infarction. The incidence was lower than in Hochberg's study but the OBS-patients were not only patients with so called acute coronary insufficiency. Further prospective studies must be done to reveal how often chest pain, considered to suggest premonitory pain, would represent a false alarm (Friedberg, 1972).

Fulton et al. (1972) in their report on the natural history of unstable angina had noticed occurrence of preceding unstable angina in 60 per cent of the 110 cases with myocardial infarction. Their study

supports the occurrence of new or changing symptoms preceding the admission to the CCU is of discriminating power between the AMI and

OBS-patients in this study in spite of the rather high incidence of prodromata even in the OBS-patients.

New or increasing chest pain, unstable angina (Fulton et al. 1972) was especially discriminating between the AMI and the OBS-group. This is also in accordance with the report of the WHO Working Group on the Prodromal Symptoms of Myocardial Infarction (1971) who expressed that only changing patterns of chest pain appear to be especially associated with acute heart attacks and therefore can be regarded as truly premonitory.

C THE ACUTE ATTACK

The day of onset

Several authors have studied the occurrence of AMI in relation to the days of week (Master 1937 b, Ströder et al. 1951, Berg et al. 1957, Jensen, 1959, Gorbатов et al. 1962, Lindholm, 1963, Askansas et al. 1970).

In the present study there was a higher morbidity during Saturday, Sunday and Monday in the AMI group than in the two other groups of patients. In 56 per cent of the AMI patients the onset of symptoms started during one of these three days against 37 per cent in the ICS-group and 42 per cent in the OBS-group. The difference in this respect between the AMI and the OBS-patients was almost significant ($p < 0.05$) (Table 24).

The lowest rate in the AMI group was noted in Tuesdays but there were no statistical differences between the three groups.

Comments

According to some authors (Berg et al. 1957, Jensen, 1959, Uhlenbruch, 1967) the risk is greatest during the weekends owing to parties or other entertainment on Saturdays, a heavy rich meal on Sundays, usually with alcohol and often very strenuous work on Mondays (Lindholm, 1963). In this study there was also a difference between the AMI patients and the OBS-patients in this respect. In one study recently published from Warsaw (Askansas et al. 1970) there was a significant trend of decrease in the AMI incidence rate starting with the first to the last day of the week (Mon-

TABLE 24 The day of onset of symptoms leading the patients to CCU. A comparison between the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
Sunday	18	23	19	—	10	5	7	21	16
Monday	20	18	19	11	20	16	12	7	10
Tuesday	9	5	8	22	10	16	19	17	18
Wednesday	11	5	11	33	10	21	9	10	10
Thursday	11	14	11	11	20	16	11	20	17
Friday	8	14	9	11	18	11	9	12	11
Saturday	15	23	17	11	20	16	23	7	16

day to Sunday). Even Master (1937 b) found a high frequency of onset of symptoms on Mondays while Gorbатов et al. (1962) and Lindholm (1963) found no statistically significant variation with the days of the week and no tendency for disease to occur during weekends.

In this study there was a slightly higher incidence of onset of symptoms during the weekends than during the other days of the week in the AMI group but not in the other two groups of patients.

The time of onset

For the present study the day was divided into four parts shown in Table 25. There are no statistical differences between the groups. Eight per cent of the AMI patients and 11 per cent of the OBS-patients could not give any information about the time of onset.

Comments

There were no significant differences between the groups concerning the time of onset in this study. This was in accordance with the findings presented in part I (page 16). Some authors have shown a higher incidence in daytime (from 8 am. to 6 pm) than in the night (from 6 pm to 6 am) (Ströder et al. 1951, Askansas et al. 1970) while others have pointed out this tendency but also emphasized that several patients cannot give the hour of onset and that most of these cases might have occurred during night (Lindholm, 1963).

The time between onset and admission to CCU

The time between onset of symptoms and admission to CCU i.e. the delay has been subjected to considerable interest in relation to CCUs (Hackitt and Cassen 1969, Moss 1969, Gilchrist 1971).

TABLE 25 Time of onset of symptoms in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 22 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
01—06	18	36	3	11	20	16	16	27	19
0 — 1		18	3	41	30	37	33	29	31
15—18	9	11	2		30	6	3	10	17
19—00	18		7		20	1	19	7	3

Armstrong et al. 1972, Hamaryt et al. 1972 Tjoe and Luria 1972 Yu 1972) The delay varies in different communities probably due to differences in the medical knowledge of the public, availability of transportation and distances to the hospital from the place of occurrence of the attack as well as other factors. An important proportion of this time is called the patient delay which is that part which is influenced by the patient's own action.

In this study as can be seen in Table 26 delay of less than 3 hours is roughly equally common in all groups, while a delay of less than 6 hours was almost significant more common in the AMI and ICS-patients than in the OBS-patients ($p < 0.05$).

More than 75 per cent of all the patients were admitted within 12 hours with similar distribution in all three groups. Eight per cent of the AMI patients and 11 per cent of the OBS-patients could not give certain information about time of onset or delay.

Comments

A delay of less than 6 hours was more common in the AMI and ICS-groups than in the OBS-group. This was in accordance to the finding presented in part I (page 16). One study presented by Engstedt et al. (1971) showed that the AMI and OBS-patients were hospitalized in about the same frequency when calculating with a delay of less than 4 hours.

Tjoe and Luria (1972) have showed that there was no statistical difference in decision times of patients with chest pain admitted to the CCU whether or not their eventual diagnosis was that of AMI.

Wikland (1971) showed, in his study that there was no striking over or underrepresentation of previous myocardial infarction, angina pectoris, heart failure, hypertension or other disorders in the group of patients who call for medical assistance during the last fatal attack of chest pain in comparison to those who did not. In order to analyze the patient delay factors a more detailed investigation is being carried out in our CCU (Erhardt et al.).

Activity at onset of symptoms

Many authors have studied the physical and mental activity at onset of symptoms in acute myocardial infarction (Master, 1937 Blumgart 1945 Billings, 1949 Yater et al. 1951 Sievers, 1964, Wikland, 1971 Robert, 1972).

The recorded activities in the patients of this study are shown in Table 27.

In the AMI-group 19 per cent of the patients were asleep at onset of symptoms and awaked with or by the symptoms. Eleven per cent of the ICS-patients and 16 per cent of the OBS-patients gave the same history.

Activities such as lying down but not sleeping, sitting and standing still were defined as at rest and reported by 14 per cent of the AMI patients,

TABLE 26 Time between onset of symptoms and admission to CCU in the three groups of patients

	AMI			ICS			OBS		
	Males = 66 per cent	Females = 12 per cent	Total = 88 per cent	Males = 9 per cent	Females = 10 per cent	Total = 19 per cent	Males = 43 per cent	Females = 41 per cent	Total = 84 per cent
< 1 hrs	12	5	10	2	0	1	9	11	6
< 3 hrs	23	10	21	11	30	32	16	12	14
< 6 hrs	65	64	65	67	70	68	49	54	51
< 12 hrs	79	82	80	89	70	79	74	76	75
> 1 hrs	91	96	92	100	100	100	91	83	89

$p < 0.05$

TABLE 7 Types of activity at onset of symptoms in the patients in the three diagnostic groups

	AMI			ICS			OBS		
	Males n = 66	Females n = 2	Total n = 68	Males n = 9	Females n = 10	Total n = 19	Males n = 43	Females n = 41	Total n = 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Sleep	1	7	19	—	20	11	1	10	16
Rest (lying or sitting)	15	9	14	11	30	1	9	27	18
Mild to moderate activity	30	46	49	67	30	38	51	49	51
Unusual or severe exertion	15	9	14	2	—	11	9	2	6
Mental stress	15	—	1	—	—	—	—	(2)	(1)
Uncertain	15	9	3	—	—	—	9	12	9

21 per cent of the ICS-patients and 18 per cent of the OBS-patients.

Normal physical and mental activities were defined as activities of ordinary daily living such as walking, personal hygiene, household work and ordinary work. About half of the patients in each of the three diagnostic groups reported such activities. There were no statistical differences in any of these respects.

Heavy physical activity at onset of symptoms was reported by 14 per cent of the AMI patients, 11 per cent of the ICS-patients and 6 per cent of the OBS-patients. These differences were not statistically significant.

One patient in the AMI group developed chest pain when he was taking part in a demonstration. Another patient in the OBS-group got her symptoms when visiting a pharmacy. These two patients themselves mentioned mental stress at the onset of chest pain.

Three AMI patients and 7 OBS-patients could not adequately define their activities at onset.

Consumption of alcohol during the last 24 hours before admission

Some patients had taken alcohol before the onset of symptoms whereas other had tried alcohol to relieve the pain after onset. The quantity of alcohol was mostly moderate and not estimated in detail.

There was a difference between the sexes in the consumption of alcohol. About a fourth of the men had had an intake of alcohol before admission against about a tenth of the women.

As can be seen in Table 28 the circumstances were about the same in the AMI and in the OBS-group. Two patients in the ICS-group had taken alcohol before admission.

Comment

The findings in this study concerning the activities of patients at onset are in accordance to previous studies of (Master 1937, Sievers 1963, Roberts 1972). There was no difference between these patients and the OBS-patients in this respect.

TABLE 28 Consumption of alcohol during the last 24 hours before admission in the three diagnostic groups

	AMI			ICS			OBS		
	Males n = 66	Females n = 2	Total n = 68	Males n = 9	Females n = 10	Total n = 19	Males n = 43	Females n = 41	Total n = 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Patients with consumption of alcohol	1	—	1	1	—	1	3	7	16

As previously shown the prevalence of onset of AMI was rather low in immediate connection with physical and mental stress (Master 1937 Billings et al. 1949 Solomon et al. 1969) Master and his associates (1937) pointed out that since half of the day of normal persons is spent in mild or moderate activity one might well expect half of all attacks to occur during this kind of activity and concluded that effort is not a precipitating factor in causing myocardial infarction. Blumgart (1945) did not agree and quoted Bean, who pointed out, the fact that although most motor accidents do not occur at speeds of 70 miles an hour does not prove that such speed may not be concerned in some motor accidents. The intake of alcohol within 24 hours before admission was the same in the AMI and OBS-patients and more common in men than in women.

To sum up the activities at onset of symptoms leading the patients to CCU are about the same in the different groups and give no help in the early diagnosis of AMI

SYMPTOMS AT ONSET

Anterior chest pain is the most prominent symptom in acute myocardial infarction (Heberden, 1786 Herrick, 1912 Bruenn 1936, Bean, 1938 Rosenbaum Levine, 1941 Chambers, 1946 Brörck 1960 b Scholmerich, 1962, Reimann and Jahrmärker 1969 Chapman, 1971 Kontinen, 1971 and Simpson and Chenkin, 1971) On the other hand there are innumerable noncardiac conditions, visceral as well as somatic that give rise to anterior chest pain (Pruzzmetal, 1955)

The incidence of so called *painless infarctions* varies from 0 per cent to more than 60 per cent

(Babey 1939 Roseman, 1954 Evans and Sutton 1956, Stokes and Dawber 1959 and Lindberg et al. 1960) and there is a tendency in fact that the number of these is decreasing with improved history taking. Better clinical diagnosis will give fewer painless infarctions (Uhlenbruck and Land 1967) It is important to make a distinction between painless and symptomless myocardial infarction (Michelmayr and Schweitzer 1967) Reimann and Jahrmärker (1969) shows in their study that 15 per cent of the AMI patients were without pain at onset but only 15 per cent without any symptoms such as dyspnoea, sweating and disturbances in consciousness. The problems of denial of chest pain have been studied during the last years (Olin and Hackett, 1964 Croog et al., 1971) It is apparent that one of the reasons for different incidences of painless infarctions is connected with this aspect. Croog et al. (1971) showed that in 345 male cardiac patients 20 per cent denied that they had had a heart attack as soon as 3 weeks after their first myocardial infarction. One year later the results were the same suggesting persistence of the denial

In the present study no judgement of the incidence of painless or symptomless infarctions could be made because of our criteria for admission to the CCU (page 11)

Pain

Table 29 shows the patients in the different diagnostic groups admitted on criteria 2—4 (page 11) Nine patients in this study were admitted without pain—3 AMI patients 1 ICS-patients and 5 OBS-patients

All other patients gave a history of chest pain

TABLE 29 Patients in the three diagnostic groups admitted on other criteria than chest pain

Cause of admission	AMI			ICS			OBS		
	Males = 2	Females = 1	Total = 3	Males = 1	Females = 1	Total = 1	Males = 4	Females = 1	Total = 5
Pulmonary oedema	1	—	1	—	—	—	2	1	3
Shock	—	—	—	—	—	—	—	—	—
Syncope	1	1	2	1	—	1	2	—	2

at onset and these have been asked in detail about the quality, localization, duration and intensity of the chest pain and about occurrence of radiation of pain to the arms or extension of the pain to other areas than the anterior chest wall. No study of anxiety at onset of chest pain was performed.

Types of chest pain

Thirtyfour per cent of the AMI patients used the expression "pain" or ache in their description. The same expressions were also used by 37 per cent of the ICS-patients and by 29 per cent of the OBS-patients. (Table 30)

A type of pain more like oppression was reported by 16 per cent of the AMI patients and 26 per cent of the ICS-patients while 37 per cent of the OBS-patients characterized their type of pain in this way. The difference between AMI and OBS-patients was significant ($p < 0.01$).

A description of a constricting or vicelike pain was given by 18 per cent of the AMI patients, 11 per cent of the ICS-patients and 7 per cent of the OBS-patients. The difference between the AMI and OBS-patients was almost significant ($p < 0.05$).

A burning or smarting pain was indicated by 14 per cent of the AMI patients, 5 per cent of the ICS-patients and 7 per cent of the OBS-patients. The difference did not reach significance.

Repeated bouts of pain relieved spontaneously or by nitroglycerine occurred in 13 per cent of

the AMI patients and 16 per cent of the ICS-patients and in 12 per cent of the OBS-patients.

Two per cent in both the AMI and OBS-group indicated discomfort in the chest as a description of their complaints.

Comments

The classification of the quality of the pain is difficult because of semantic difficulties (Fenster, 1969 and Boyle 1970). The patient's description is built upon previous experiences, different vocabulary and imagination. Several authors have tried to analyze the types of pain in AMI (Bean, 1938; Prinzmetal and Massumi 1955; Keele, 1968 and Sampson and Chertlin, 1971). The descriptions vary greatly with the countries since the words used are often not easily translatable.

In this study it is shown that chest pain of constricting or vicelike character is somewhat more common in AMI patients while pain described as a pressure is more common in the OBS-patients. The discriminative power of these differences is small since the semantic problems are complicated in this field of the patient-doctor relationship. This is also the reason why no attempt has been made to evaluate the occurrence of anxiety at onset of chest pain. Plügge (1935) has tried to clarify the distinction between chest pain and other types of pains e.g. renal colic. The latter is located peripherally to an organ and can be objectively looked upon by the patient while chest pain interpreted

TABLE 30 Types of chest pain in the three diagnostic groups

	AMI			ICS			OBS		
	Males n = 31	Females n = 2	Total n = 33	Males n = 9	Females n = 10	Total n = 19	Males n = 43	Females n = 41	Total n = 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Painful	3	5	3	11	—	3	9	2	6
Pain, ache	53	36	34	33	40	37	26	32	29
Pressure	1	5	16	2	30	6	33	41	37
Constriction	15	—	18	2	—	11	7	7	7*
Burn	5	9	14	—	10	5	12	2	7
Staccato anginous	—	—	13	11	20	16	14	10	12
Discomfort	5	—	—	—	—	—	—	5	—

$p < 0.05$ $p < 0.01$

as cardiac pain is more central more life threatening and existential in its character. If this difference of experience is biological or a product of empirical knowledge about the connection between cardiac pain and death is a question of philosophy according to Plügge.

Börck (1967) considered that the anxiety can be a part of the experience of chest pain, but also an independent psychological reaction associated with the conception that this symptom is an ominous danger signal.

Localization of the pain over the chest

The patients were asked to demonstrate the area of pain over the chest by tracing it with their fingers. The result was registered with regard to site: retrosternally parasternally left or right, retroparasternally or para-retroparasternally (Säwe 1971).

Pain mainly retrosternally was indicated by 25 per cent of the AMI-patients, 32 per cent of the ICS-patients while 12 per cent of the OBS-patients gave this information. This difference was almost significant ($p < 0.05$) (Table 31). Concerning the other sites there were no differences between the three groups.

Only one per cent of the AMI and of the OBS-patients had pain only to the right of the sternum. Pain localized from the left to the right medioclavicular line over the chest was indicated by 45 per cent of the AMI patients, 32 per cent

of the ICS-patients and 40 per cent of the OBS-patients.

Extension of chest pain to other areas

Pain extending to the neck was found in 14 per cent of the AMI patients, in 32 per cent of the ICS-patients and in 13 per cent of the OBS-patients (Table 32).

Ten per cent of the AMI patients had pain extending to the jaw while none of the other patients mentioned this extension ($p < 0.01$).

Three per cent of the AMI-patients, 16 per cent of the ICS-patients and 5 per cent of the OBS-patients felt an extension of the chest pain to the shoulders.

In 15 per cent of the AMI patients there was a less well defined pain in the back in combination with the chest pain. This was also found in 5 per cent of the ICS-patients and in 18 per cent of the OBS-patients.

Pain in the back was more common in women than in men regardless of final diagnosis. Ten per cent of the men had pain in their back against 23 per cent of the women ($p < 0.05$).

Some of the patients indicated extension of the pain to several areas.

Radiation of pain to the arms

Radiation of pain to the arms is a referred pain and implies that the patient has an experience of pain in an area separated from the organ which

TABLE 31 Localization of the pain over the chest in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66	Females = 22	Total = 88	Males = 9	Females = 10	Total = 19	Males = 43	Females = 41	Total = 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Painless	3	5	3	11	—	3	9	2	6
Retrosternally	26	23	23	11	30	32	8	15	12
Parasternally left	15	9	14	11	—	5	26	20	23
Retroparasternally	8	23	11	33	20	26	14	22	18
Parasternally right	2	—	1	—	—	—	—	2	1
Para-retroparasternally	47	41	45	33	30	32	42	39	40

$p < 0.05$

while 19 per cent of the OBS-patients indicated this duration of pain. This difference was significant ($p < 0.01$) (Table 34).

More than half of the AMI patients and half of the ICS-patients had a duration of pain between one hour and six hours while a third of the OBS-patients indicated this duration. This difference was almost significant ($p < 0.05$).

A duration of pain more than six hours did not significantly separate the groups. It was indicated by 41 per cent of the AMI-patients, 39 per cent of the ICS-patients and 46 per cent of the OBS-patients.

Morphine given on admission

Morphine was given to 67 per cent of the AMI patients and 47 per cent of the ICS-patients while 37 per cent of the OBS-patients was given this on admission. The difference between the AMI and OBS-patients was highly significant ($p < 0.001$) (Table 34).

Comments

Yater et al. (1948) have shown that only 8 per cent of the AMI patients in their material had a duration of pain less than one hour. In that study 30 per cent of the AMI patients marked a duration of pain between one and four hours while 55 per cent indicated pain from 4 to 48 hours.

The conclusion of the present study was that a duration of pain less than one hour but more

than 15 minutes (see admission criteria page 11) speaks against the AMI-diagnosis while a duration between one and six hours speaks for this diagnosis and a duration of more than six hours was less specific.

The intensity of pain is difficult to estimate. In this study the AMI patients and the ICS-patients were given morphine more often on admission than the OBS-patients. Meltzer (1968) said that 62 per cent of the AMI patients had moderate to severe pain during the attack. The quantification of pain is however very difficult since the pain threshold is different in different individuals. Many authors have therefore tried to subdivide the patients in different groups of sensitiveness with different algometric methods (Libman, 1934). Keele in his report (1968) considered that 23 per cent of the patients were hypersensitive according to his definition. Sixty-two per cent were normosensitive and 15 per cent hyposensitive. He claimed that the pattern of pain was probably a function of the individual threshold and the noxious stimulation.

Dyspnoea

Several authors have pointed out that dyspnoea is next to pain the most salient symptom at onset of an acute myocardial infarction (Bean, 1938; Rosenbaum-Levine 1941; Billings et al., 1949 and Schölménch, 1962) furthermore, in some patients

TABLE 34 Duration of pain in the patients with this symptom before admission, and the administration of morphine on admission in the three diagnostic groups

	AMI			ICS			OBS		
	Males n = 64 per cent	Females n = 31 per cent	Total n = 95 per cent	Males n = 8 per cent	Females n = 10 per cent	Total n = 18 per cent	Males n = 39 per cent	Females n = 40 per cent	Total n = 79 per cent
Less than 1 h	8	—	8	—	10	6	18	20	19 ^a
1—6 hrs	—	6	55	63	30	56	33	38	33
More than 6 hrs	—	33	41	37	40	38	49	4	46
Morphine given on admission	—	3	—	44	40	4	33	39	37
$p < 0.01$	$p < 0.01$	p							

TABLE 35 The incidence of dyspnoea before admission to the three diagnostic groups

	AMI			OBS		
	Males n = 66	Females n = 33	Total n = 99	Males n = 43	Females n = 41	Total n = 84
	per cent	per cent	per cent	per cent	per cent	per cent
No dyspnoea	61	54	5	6	63	46
Dyspnoea	36	32	3	44	44	44
Rattling respiration	5	14	—	9	10	10

with AMI dyspnoea can be the only symptom (Master et al., 1937; Roseman, 1954). Bjerk and 1957 on the other hand, maintains that subjective impressions of dyspnoea during the acute attack of pain cannot be judged because patients so often confuse dyspnoea with the chest pain or the feeling of oppression that accompanies the pain.

In the present study dyspnoea is defined as a symptom indicated by the patient and not a sign registered by the observer. The dyspnoea was characterized by the patients as shortness of breath or as if they were out of breath. A few patients experienced rattling respiration.

As can be seen in Table 35 35 per cent of the AMI patients had experienced dyspnoea before admission and a further 7 per cent rattling respiration. Dyspnoea was indicated by 37 per cent of the

ICU-patients but none of them indicated rattling respiration. In the OBS-group 44 per cent had felt difficulties in breathing and 10 per cent felt rattling respiration.

The occurrence of previous heart failure and history of smoking in the patients with or without dyspnoea are shown in Table 36, where also objective signs such as tachypnoea and rales at admission are presented.

A history of heart failure was obtained more often in the dyspnoea groups with a very high incidence in the group with rattling respiration. Previous heart failure was more common in the OBS-group with dyspnoea than in the corresponding AMI group ($p < 0.05$).

As has been shown the patients in the AMI group smoked more than the patients in the other

TABLE 36. The symptom of dyspnoea compared with some objective parameters in the history and the physical examination of the 191 patients in the three diagnostic groups

	AMI			ICS			OBS		
	No dyspnoea n = 51	Dyspnoea n = 31	Rattling respiration n = 6	No dyspnoea n = 12	Dyspnoea n = 7	Rattling respiration n = 0	No dyspnoea n = 39	Dyspnoea n = 37	Rattling respiration n = 8
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Previous heart failure	26	39*	100	67	71	—	39	63	75
Smoker	51	65	53	111	14	—	15	32	38
Respiratory rate > 20	75	51	33	8	79	—	23	27	87
More than few rales at the lungs	28	48**	83	25	14	—	13	16	100

* $p < 0.05$ ** $p < 0.01$

groups (page 38) There were, however, no statistical differences between smokers and nonsmokers concerning dyspnoea at onset of symptoms.

Tachypnoea on admission, here arbitrarily defined as a respiratory rate of $>20/\text{min.}$ occurred in 34 per cent of the AMI-patients complaining of dyspnoea against 27 per cent of the corresponding OBS-patients, and 29 per cent of the ICS-patients with dyspnoea. The difference between the AMI and OBS-patients in this respect was almost significant ($p < 0.05$).

A presence of more than a few scattered basal rales on admission was noted in 48 per cent of the AMI patients with dyspnoea against 16 per cent of the corresponding OBS-patients and 14 per cent of the ICS-patients. The difference between the AMI and the OBS-patients was significant ($p < 0.01$).

Nearly all of the patients with rattling respiration had a history of previous heart failure and had rales on the lungs on admission. Two of the AMI patients with rattling respiration had a respiratory rate of more than $20/\text{min.}$ against 7 of the corresponding OBS-patients.

Comments

In the present study dyspnoea is defined as a symptom. This symptom occurred in about the same frequency in the three diagnostic groups. This corresponds to the findings presented in part I (page 17). The subjective difficulty in breathing is often associated with a feeling of constriction or being out of breath. In some patients the dyspnoea is the first and sometimes the only symptom. In other patients the difficulty in breathing appears later on. Liberman (1931) postulated that severe pain may cover dyspnoea and on the other hand, marked dyspnoea may cover a moderate amount of pain.

The similar frequency of dyspnoea in the three diagnostic groups speaks for a multifactorial connection between dyspnoea and other symptoms leading a patient to the hospital.

There were however some objective differences between the three groups of patients. Both tachypnoea and occurrence of basal rales on admission were

more common in the AMI patients indicating dyspnoea than in the corresponding OBS-patients.

In the patients of the OBS-group with dyspnoea a history of previous heart failure was more common than in the corresponding AMI-patients.

There were no statistical differences between smokers and nonsmokers concerning dyspnoea at onset.

There are several explanations for this symptom in these patients (Nilzell 1967 Rapaport, 1971). One is acute left heart failure secondary to the heart damage (Bean, 1938). But even Christman (1923) pointed out the disproportion between the degree of dyspnoea and any abnormalities demonstrable in the heart and lungs.

Dyspnoea can be a symptom of cardiac neurosis (Björck 1937) with hyperventilation in combination with severe pain and anxiety (Moia, 1971). A third explanation can be the occurrence of acute left heart failure without acute necrosis of any part of the heart.

The different figures presented in the literature may depend on different definitions. Some authors define dyspnoea as a symptom and a sign at the same time while other authors mean only a symptom or a sign. Björkelund (1937) only considered dyspnoea in cases which on examination had objective signs of dyspnoea or in cases without simultaneous pain.

Bean (1938) showed a incidence of 95 per cent with dyspnoea. In his study more than 25 per cent of the patients had Cheyne Stokes respiration. More than 70 per cent of the AMI patients were reported having dyspnoea by Rosenbaum-Levine (1941) Chambers (1946) and Billings et al. (1919). In a Swedish study recently published (Henning and Holmberg 1971) the frequency of dyspnoea and rattling respiration were similar to the findings in the present study concerning the AMI patients.

To sum up dyspnoea is not an useful differential diagnostic parameter for purpose of early evaluation of patients with onset of central chest pain. Some objective signs such as occurrence of basal rales or high respiratory rate in connection with

dyspnoea can, however, increase the validity of this symptom as connected with acute heart damage.

Disturbances in consciousness

The interpretation of symptoms varies with the clarity of perception in each individual (Bean 1938). Sudden clouding of the sensorium or unconsciousness can in rare cases be the only symptom of AMI (Chilazi 1967, Eriksson 1970, Bostrom and Ström, 1971, Carré 1971).

A distinction between clouding of the sensorium or fainting and syncope or unconsciousness was made. As can be seen in Table 37 9 per cent of the AMI-patients had experienced some clouding of the sensorium and 6 per cent had been unconscious. In the ICS-group 37 per cent of the patients indicated disturbances in consciousness and 5 per cent (1 patient) unconsciousness while 19 per cent in the OBS-group had a history of fainting prior to the admission to CCU and 11 per cent had been unconscious. The difference between the AMI and ICS-patients was significant ($p < 0.01$) and between the AMI and OBS-patients almost significant ($p < 0.05$). If the AMI and ICS-patients together were compared to the OBS-patients there was no statistical difference.

Comments

In this study the incidence of fainting and unconsciousness before admission to the CCU was

more pronounced in the ICS- and OBS-group than in the AMI group. Patients with cerebral lesions, alcoholism, diabetic coma or certain active infections may sometimes not adequately register the symptom of an AMI because of a low perception (Bean 1938). This is also the fact in psychotic patients (Wolpert et al. 1971).

In the present study only patients with a known onset of symptoms within 48 hours are included. The description of the disturbances of consciousness vary but most of the patients said that everything went black or grew dim. Some patients felt a lightheadedness. The periods of unconsciousness were usually reported as being of rather short duration.

The similar incidence of fainting or unconsciousness in the three diagnostic groups indicates grounds to suspect different causes in these patients.

Severe pain, emotional stress and vasovagals reactions can be the cause of fainting and syncope at onset of the symptoms leading to hospitalisation (Rushmer 1955, Shillingford 1970). Disturbances of heart rhythm can probably explain some of the episodes of a feeling of fainting.

A decrease in heart rate and cardiac output with an associated reduction in peripheral resistance and blood pressure can be the cause of syncope in the AMI-patients due to the acute heart damage. The similarity to the OBS-patients in this respect may however, motivate a suspicion that autonomic mechanisms are activated at the onset of chest

TABLE 37 The incidence of disturbances of consciousness in the three diagnostic groups

	AMI			ICS			OBS		
	Males = 66	Females = 22	Total = 88	Males = 9	Females = 10	Total = 19	Males = 43	Females = 41	Total = 84
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Intact sensorium	86	82	85	44	70	58	72	68	70
Clouding of sensorium	9	9	9	44	30	37	19	20	19
Unconsciousness	5	9	6	11	—	5	11	12	11

The difference between the AMI and ICS-patients is significant ($p < 0.01$) and between the AMI- and OBS-patients almost significant ($p < 0.05$).

TABLE 38 Palpitations correlated to heart rate, blood pressure, pulse pressure and occurrence of atrial fibrillation (AF) on admission and of previous treatment of digitalis in the three diagnostic groups.

		Heart rate		Blood pressure		Pulse pressure		Rhythm	Digitalis	
		Syst		Diast					No	Yes
		<60/ min	>100/ min	>160/ mm Hg	>93/ mm Hg	<40/ mm Hg	>70/ mm Hg	AF		
AMI										
No palpitation	n = 73	10	22	33	39	11	16	7	50	23
Palpitation	n = 15	—	6	5	8	3	3	2 _{aa}	6 ^a	9 ^a
ICS										
No palpitation	= 12	—	3	8	3	2	6	1	5	7 ^a
Palpitation	= 7	—	1	4	3	1	3	1	1	1
OBS										
No palpitation	= 41	2	13	23	12	1	16	5	19	23
Palpitation	= 40	2	19	21	12	3	13	13 _{aa}	20	20

^{aa} $p < 0.01$

pain and anxiety and that this is the most common cause of fainting and syncope in most of the patients.

This symptom therefore gives no diagnostic aid in the early diagnosis of the patients admitted to the CCU as stated in part I. The disturbances of

as are reported in different series of patients from 3 to 11 per cent (Chambers 1946, Bean 1938) with several authors indicating about 10 per cent (Billings et al., 1949 Reumann

1969 Verdun de Cantogno 1971) Henning and Holmberg (1971) give an incidence of 26 per cent in their study from Gothenburg, Sweden.

Arrhythmic sensations

Arrhythmic sensations were more common in the OBS-patients described in part I (page 18) as compared to the AMI patients ($p < 0.001$) There was also a difference between the sexes in this respect regardless of final diagnosis ($p < 0.001$)

TABLE 39 Arrhythmic sensation before admission reported by the three diagnostic groups

	AMI			ICS			OBS		
	Males n = 66 per cent	Females n = 22 per cent	Total n = 88 per cent	Males n = 9 per cent	Females n = 19 per cent	Total n = 28 per cent	Males n = 43 per cent	Females n = 41 per cent	Total n = 84 per cent
Rapid regular	5	9	6	2	10	16	7	15	11
Rapid irregular	—	18	6	11	—	5	23	1	11
Extra beats	5	9	6	11	18	11	12	22	17
Slow regular or irregular	—	—	—	—	10	3	2	2	2
Arrhythmic sensation of any kind	1	4	18	44	30	37	41	51	48

$p < 0.01$ $p < 0.001$

In this part of the study an attempt has been made to correlate these subjective sensations to some objective parameters (Table 38)

The arrhythmic sensations were described as a feeling of a rapid regular or irregular rhythm, slow regular or irregular rhythm or extra beats

Eighteen per cent of the AMI-patients gave a history of this kind following the onset of symptoms (Table 39). In the ICS-group the incidence of arrhythmic sensations was 37 per cent and in the OBS-group 48 per cent. The difference between the AMI and OBS-group was highly significant ($p < 0.001$) as was the difference in this respect between the sexes regardless of final diagnosis ($p < 0.001$). Even the difference between sexes within the AMI-group was significant ($p < 0.01$). The differences between the AMI and ICS-patients and between the ICS- and OBS-patients were not significant.

As can be seen in Table 38 only two of the 9 AMI-patients with atrial fibrillation on admission had felt any arrhythmic sensations against 13 of the 16 OBS-patients with this electrocardiographically verified arrhythmia on admission. One of the two ICS-patients with atrial fibrillation on admission had experienced palpitations. The difference between the AMI and OBS-patients was significant in this respect ($p < 0.01$).

Patients with any type of tachycardia ($>100/\text{min}$) had arrhythmic sensations somewhat more often regardless of final diagnosis ($p < 0.05$).

Patients with previous treatment with digitalis had higher incidence of palpitation in the AMI and ICS-groups ($p < 0.01$) but not the OBS-group.

No correlation was found between palpitation and a systolic blood pressure above 160 mmHg, a diastolic above 95 mmHg or a pulse pressure above 70 mmHg.

Comments

As already shown in part I (page 18) the difference in arrhythmic sensations was highly significant between AMI and OBS-patients. The patients in the ICS-group seemed to have incidence of palpitation which falls between the two other

groups. The difference in the experience of palpitation between men and women is highly significant.

It is generally accepted that the main reason for the mortality reduction in the CCUs is improved diagnosis and treatment of cardiac arrhythmias (Lown et al. 1967, Kullip 1968, Meltzer 1968, Sloman 1968, Pantridge and Adgey 1969, Hofrenstahl 1971) and it is probable that in the great majority of the very early deaths in AMI, arrhythmias are the responsible mechanism (Wiklund 1972). For these reasons perhaps more intensive attention ought to be given to the symptoms of arrhythmic sensations.

As has been shown in the present study the AMI patients' subjective registration of the action of the heart at the attack corresponds badly to the knowledge of a high incidence of arrhythmias during the initial stage of AMI. This was pointed out already by Bean (1938) but no certain explanation of this has been offered. The palpitation threshold suggested to be high in a flegmatic mentality, thick chest wall and high age and low at anxiety, catechol release and youth (Harrison-Reeves 1968). None of these findings is suggested to be of importance as an explanation of the difference between patients with or without AMI. The occurrence of a high systolic or diastolic pressure was about the same in the different groups and gave no clues. A pulse pressure above 70 mmHg was associated with about the same incidence of arrhythmic sensations as one below 70 mmHg.

In the present series the AMI and ICS-patients on digitalis at time of onset of symptoms more often felt arrhythmic sensations than the corresponding patients without. In contrast no such difference was found in the OBS-patients.

A plausible reason for the discrepancy concerning arrhythmic sensations in patients with and those without AMI could be the more severe pain in the AMI-patients which are apt to minimize the importance of less obvious symptoms.

Any certain explanation for the difference in experience of arrhythmic sensations cannot be given and further investigations in this field ought to be done.

Billings et al. (1949) found arrhythmic sensations in 7 per cent and Henning and Holmberg (1971) gave a higher figure of 26 per cent. Several authors have pointed to a high incidence of palpitation in patients with cardiac neurosis (Björck 1957 Mjosa 1971 Furberg 1971) v Alzenhofer (1971) has noticed that even patients with cervical spondylitis may feel arrhythmic sensations more readily probably because of facilitation in the central nervous system and Mann (1970) has pointed out that disturbances of cardiac rhythm and digestion appear together.

To sum up, the noteworthy discovery of a higher incidence of arrhythmic sensations in patients admitted to the CCU without subsequently verification of the AMI-diagnosis as compared to those with a final AMI-diagnosis reported in the series presented in part I (page 18) was confirmed in this prospective study.

Autonomic Symptoms

The patients admitted to the CCU often indicated autonomic symptoms. As has been shown in part I (page 19) there are reasons for separating nausea, or feeling sick, from vomiting and/or sweating. Since one of the causes for the auto-

nomous symptoms especially that of feeling a desire to vomit, nausea, could be intoxication of digitalis (Lely and van Enter 1972) an analysis of the patients has been made with this in mind as can be seen in Table 40. Morphine ought to be of a less importance since the patients in Stockholm with symptoms suggestive of an AMI commonly are referred to hospital by an ordinary ambulance at once without waiting to see a physician.

Nausea

The Swedish word "illamående" corresponds roughly to the latin word *nausea* and stands for feeling sick or feeling a desire to vomit. This vague symptom occurred as only autonomic symptom in 5 per cent of the AMI patients, 11 per cent of the ICS-patients and 13 per cent of the OBS-patients. The difference between the AMI and OBS-patients was almost significant ($p < 0.05$). Fourteen of the 17 patients indicating this symptom were on digitalis therapy.

Nausea and sweating

Nausea with sweating occurred in 15 per cent of the AMI-patients and 20 per cent of the OBS-patients while none of the patients in the ICS-

TABLE 40 Autonomic symptoms at onset correlated to previous treatment of digitalis in the three diagnostic groups

	n	Nausea per cent	Nausea + Sweating per cent	Vomiting per cent	Vomiting + Sweating per cent	Sweating per cent
AMI						
Total	89	5	15	5	18	30
no digitalis	46		16		1	32
digitalis	42	9	13	—	15	25
ICS						
Total	19	11	—	5	16	26
no digitalis	6	—	—	—	—	30
digitalis	13	15	—	8	23	15
OBS						
Total	4	13	20	—	11	19
no digitalis	2	0	1	5	5	51
digitalis	2	0	20	13	16	9

The difference between patients with nausea and without digitalis is significant ($p < 0.01$)

group reported this constellation of symptoms. There was no difference between patients with or without digitalis in this respect.

Vomiting

Vomiting with or without a previous feeling of a desire to vomit and without sweating was indicated by 5 per cent of the AMI patients, 5 per cent of the ICS-patients and 11 per cent of the OBS-patients. None of these AMI patients had digitalis while 6 of the 7 OBS-patients were on this therapy. There were no statistical differences.

Vomiting and sweating

Vomiting and sweating in combination occurred in 18 per cent of the AMI-patients, in 16 per cent of the ICS-patients and in 11 per cent of the OBS-patients. There was no statistical difference between undigitalized and digitalized AMI and OBS-patients.

Sweating

Sweating as an isolated autonomic symptom was reported by 30 per cent of the AMI patients and 26 per cent of the ICS-patients while 19 per cent of the OBS-group indicated this symptom. Sweating alone was more common in undigitalized OBS-patients than in OBS-patients with digitalis ($p < 0.05$) while there were no such differences in the other two diagnostic groups.

Combinations of autonomic symptoms

Nausea with sweating, vomiting with or without sweating and sweating alone are the constellations of symptom called combination of autonomic symptoms in part I (page 19). This was indicated by 68 per cent of the AMI patients in the present material against 47 per cent of the ICS-patients and 58 per cent of the OBS-patients. The difference between AMI and OBS-patients was not significant. Combinations of autonomic symptoms was a little more common in the undigitalized patients in the AMI-group than in the patients with digitalis ($p < 0.05$). If the constellation of nausea and sweating was excluded there was an almost significant difference between the AMI and OBS-patients ($p < 0.05$).

Comments

Autonomic symptoms in the form of nausea, vomiting and sweating are common in patients admitted to CCU (part I, page 19). In the present study the different autonomic symptoms have been studied separately.

Patients indicating vomiting have mostly felt nausea before and/or after the vomiting. As demonstrated in part I nausea or feeling sick as an isolated symptom was more common in the OBS-patients than in the AMI patients. Most of the patients with this single symptom were on digitalis.

Vomiting, sweating and combination of these two symptoms occurred more often in the AMI patients than in the OBS-patients. With the exception of a slightly higher prevalence of sweating in undigitalized OBS-patients than in OBS-patients with digitalis there were no differences between patients with or without digitalis.

There are several conceivable explanations of autonomic symptoms in the patients admitted to CCU. Digitalis intoxication can be one (Lely and van Enter, 1972) whereas morphine ought to be of a less importance since only a few of the patients in the present study had seen a physician before admission to the hospital.

Nausea, or feeling sick is a symptom of a more psychological nature according to Wang and Bonason (1950-1953) while vomiting is more complicated symptom (Harrison et al 1965). Vomiting stimuli fall into two groups: those acting primarily by enhancing the activity of the vomiting center (central vomiting) and those arising in the peripheral part of the body and conveyed to the vomiting center (reflex vomiting). Both nausea and vomiting can be due to heart failure and the hepatic and gastrointestinal congestion secondary to failure. Hatcher and Weiss (1927) proposed the theory that autonomic symptoms in acute myocardial infarction were due to strong medullary bombardment of impulses from the heart.

The incidence of autonomic symptoms at the early stage of AMI is generally rather high in different series. Bean (1938) recorded an incidence

pressure was highly significant lower in the AMI group, thus giving a discriminating factor in the early judgement of the patients on admission to the CCU

Heart rate on admission

Table 42 shows the heart rate and rhythm in the patients on admission. A sinus rhythm below 60/min. was noted in 11 per cent of the AMI patients and in 6 per cent of the OBS-patients while none of the ICS-patients had this heart rate. A sinus rhythm above 100/min. was registered in 22 per cent of the AMI patients and 19 per cent of the OBS-patients against 11 per cent of the ICS-patients.

One AMI patient and 2 OBS-patients had atrial flutter with a heart rate above 140. Three OBS-patients had a permanent pacemaker.

Atrial fibrillation on admission was noted in 9 AMI patients, 2 ICS-patients and 16 OBS-patients.

Comments

The heart rate and rhythm on admission were

TABLE 42 Heart rate and rhythm on admission in the three diagnostic groups

	AMI n = 88 per cent	ICS n = 19 per cent	OBS n = 81 per cent
Sinus rhythm			
< 50	5	—	1
50—59	8	—	5
60—69	10	3	21
80—99	35	4	30
100—119	15	11	15
120—159	5	—	2
> 160	—	—	2
Pacemaker	—	—	4
Atrial fibrillation			
< 100	—	—	3
100—119	—	—	—
120—159	—	—	6
> 160	—	—	1
Atrial flutter			
> 140	—	—	—

TABLE 43 Respiratory rate on admission in the three diagnostic groups

	AMI n = 88 per cent	ICS n = 19 per cent	OBS n = 81 per cent
Respiratory rate			
< 16	5	5	21
16	19	21	18
18	10	5	12
20	30	53	18
22—24	21	11	13
≥ 26	16	5	18

** $p < 0.001$

of no aid in differentiating the patients in the three diagnostic groups.

Respiratory rate on admission

In the present study only 5 per cent of the AMI patients and 5 per cent of the ICS-patients had a respiratory rate below 16/min against 21 per cent of the OBS-patients. The difference between the AMI patients and the OBS-patients was highly significant ($p < 0.001$). An increased respiratory rate above 20/min was noted in 37 per cent of the AMI patients, in 16 per cent of the ICS-patients and in 31 per cent of the OBS-patients. There was no statistical difference in this respect between the AMI and OBS-patients (Table 43).

Pulmonary rates on admission

In more than a third of the AMI patients and a third of the ICS-patients there were more than a few scattered basal rales at the lungs on admission, while this sign was found in a fourth of the OBS-patients ($p < 0.05$) (Table 44).

Comments

A low respiratory rate < 16/min. on admission speaks against the AMI-diagnosis but a respiratory rate above 20/min. does not discriminate between the three diagnostic groups. In the AMI and ICS-groups basal rales occur more often than in the OBS-group suggesting a higher left ventricular filling pressure in the former two groups.

TABLE 44 The prevalence of pulmonary rales or oedema on admission in the three diagnostic

	AMI = 88 per cent	ICS = 19 per cent	OBS = 84 per cent
No or few basal rales	61	63	77
More than few basal rales	36	37	17
Frank pulmonary oedema	2	—	6

$p < 0.05$

SENSELESSNESS ON ADMISSION

Two AMI-patients were unconscious on admission and 4 were mentally confused. Two ICS-patients and 4 OBS-patients were also mentally confused on admission. There were no differences between the groups.

The interviews of these patients were done later when they had recovered their mental faculties or with the assistance of a relative.

E. TESTS ON ADMISSION

Enzyme determinations on admission

A detailed analysis of the enzyme determinations in the patients in the present series is presented in part III (page 26). The normal range for the S-GOT values is 10–35 U/l in this hospital and 10–50 U/l for CPK (Bergström and Sävje, 1972).

About half of the AMI patients had a S-GOT value above 30 U/l on admission while none in the ICS-group had S-GOT value above this limit. Fifteen per cent of the OBS-patients had also an elevation above 30 U/l of the S-GOT on admission. The difference between the AMI patients and the other two groups was highly significant ($p < 0.001$).

A CPK value above 50 U/l on admission was found in 44 per cent of the AMI-patients but also in a fourth of both the ICS- and OBS-patients. The difference between the AMI and the OBS-patients was highly significant ($p < 0.001$) and between the AMI and the ICS-patients significant ($p < 0.01$).

Comments

An elevated value of S-GOT or CPK on admission supports the AMI-diagnosis. About half of the AMI patients had a slight elevation of the enzymes on admission but even some of the OBS-patients had this. The conclusion must be that an elevated S-GOT or CPK value on admission does not necessarily lead to a subsequent AMI diagnosis nor does a normal value on admission exclude the diagnosis. The enzyme value in an AMI depends on the time between onset of symptoms and the sampling. There is a certain loss of time in the laboratory procedures meaning that the enzymatic test is not especially valuable as a good discriminative factor in the Casualty Department.

ECG on admission

A third of the AMI-patients had signs suggestive of an AMI according to the Minnesota code on the admission ECG. None of the patients in the other groups showed this, ($p < 0.001$) but nearly all of the ICS-patients and 75 per cent of the OBS-patients had ECG with signs of previous infarction, branch bundle block or non-characteristic ST-T-changes. Only a fourth of the OBS-patients and a tenth of the ICS-patients had normal admission ECG while none of the AMI patients had this on admission. The difference between the AMI patients and the OBS-patients was highly significant ($p < 0.001$) and between AMI and ICS-patients almost significant ($p < 0.05$).

Comments

An admission ECG suggestive of an AMI is of course a useful aid in the early diagnosis of AMI. Many of the patients admitted to the CCU have pathologically changed ECG on admission and this may complicate the early evaluation of the ECG. Short (1970) has shown that in the early stages of slight or subacute myocardial infarction, classical pattern such as major ST elevation, abnormal Q waves and obvious ST and T wave changes are seen in only a minority of cases, and he stated that the cardiogram must be interpreted together with the full clinical picture.

SUMMARY

An analysis of 191 patients was performed in order to try to improve the early diagnostic accuracy in patients admitted to a CCU.

A detailed questionnaire was constructed to cover systematically prodromal symptoms and acute symptoms and events leading to hospitalisation. Furthermore, the history of previous diseases was included as were some simple clinical parameters on admission.

The incidence of previous angina pectoris and myocardial infarction was about the same in the AMI and OBS-groups while it was considerably higher in the ICS-group. Previous hypertension was as common in all three groups of patient. The occurrence of diabetes mellitus was high in the ICS-group compared to the other two groups. Peptic ulcer, renal calculus and gall stone were common previous conditions in the patients regardless of the final diagnosis.

The incidence of a positive history of smoking was higher in the AMI-group than in the two other groups. This was the case in spite of a high incidence of IHD-manifestations even in these two latter groups.

New or increasing chest pain, unstable angina, was especially discriminating between the AMI and the OBS-group.

In the AMI group there was a slightly higher onset of the symptoms during the weekends than in the two other groups. A delay of less than 6 hours was more common in the AMI and ICS-groups than in the OBS-group. The activities at onset of symptoms were about the same in the different groups.

In the present study an analysis of different parameters of the chest pain has been made. The problems concerning the occurrence of painless and symptomless infarction have been discussed.

Constructing or ice-like pain is more common in the AMI patients while pain more like an oppression more often occurred in the OBS-patients. The localization of the pain over the chest was about the same in all three groups of patients. Ten per cent of the AMI patients indicated exten-

sion of the pain to the jaw while none of the other patients mentioned this. Radiation of the pain to the arms was much more common in the AMI and the ICS-patients than in the OBS-patients. A short duration of pain, less than one hour was more often indicated by the OBS-patients than by the other two groups of patients.

Dyspnoea or disturbances of consciousness were not useful differential diagnostic parameters for purpose of early evaluation of patients with onset of central chest pain since it occurred with about the same frequency in all three diagnostic groups.

The noteworthy discovery of a higher incidence of arrhythmic sensations in patients admitted to the CCU without subsequent verification of the AMI diagnosis as compared to those with a final AMI diagnosis reported in the series presented in part I was confirmed in this prospective study.

The incidence of autonomic symptoms is high in patients admitted to the CCU. Nausea, or feeling sick as an isolated symptom occurred more often in the OBS-patients especially in those with digitalis, while vomiting and/or sweating were more common in the AMI patients.

Three patients—all in the AMI group—reported hiccups.

In the present series blood pressure, heart and respiratory rate and occurrence of basal rales have been registered on admission and ECG and enzyme-determinations have been performed on admission to the CCU.

There were no significant differences concerning the systolic or diastolic pressure on admission between the three groups of patients but a pulse pressure below 60 mm Hg was more common in the AMI group than in the two other groups.

The heart rate and rhythm on admission were of no aid in the early differentiation of the patients in the three diagnostic groups.

A low respiratory rate on admission, less than 16 per minute, was more often registered in the OBS-patients than in the two other groups of patients.

About half of the AMI patients had a slight elevation of the 5-GOT value on admission but

even a few of the OBS-patients had this. Since there is a certain loss of time in the laboratory procedures the enzymatic test is not especially valuable as a good early discriminative factor in the Causality Department.

A third of the AMI patients had signs suggestive of an AMI on the admission ECG and none of the patients in the two other groups.

These findings are the basis of the attempt to work out a diagnostic index presented in part V

Diagnostic Index based on the Early Clinical Picture

The purpose of this section of the present study is to try to construct a better clinical diagnostic index than that described in part II (page 21)

The early clinical picture described in the patients presented in part IV was the basis of this attempt.

MATERIALS AND METHODS

The 191 patients presented in part IV were divided in two groups. One group was made up of the 88 AMI patients and the 19 ICS-patients and another of the 84 OBS-patients.

Nine parameters significantly discriminating the 107 patients in the former group from the 81 patients in the latter group were used in the discriminant function analysis. From the history and the acute symptoms the following parameters were chosen: history of smoking, history of unstable angina pectoris, radiation of pain to the arms, extension of pain to the jaw, a duration of pain shorter than one hour and sensations of arrhythmia.

From the physical examination a pulse pressure below 60 mm Hg and a respiratory rate below 16 per minute were chosen as discriminating factors.

TABLE 43 Discriminant function coefficient derived from the discriminant function analysis

+0.0011	history of smoking	+ 4
+0.00196	unstable angina	+ 6
+0.00116	radiation to the arm	+ 5
+0.0073	extension of pain to the jaw	
-0.00109	duration of p in ≤ 1 hour	5
-0.0019	arrhythmic sensation	6
+0.00159	pulse pressure < 60 mm Hg	
-0.00159	respiratory rate < 16 min	8
+0.01114	admission ECG suggestive of AMI	11

An admission ECG suggestive of an AMI is an important early discriminating factor and this parameter was also included.

Dizziness as a prodrome was judged as vague, as was the type of chest pain at onset and these two parameters were excluded from the analysis.

The enzyme value on admission as a discriminating factor was excluded since there is a certain loss of time in the laboratory procedures.

The same statistical methods as described in part II are used (page 21)

RESULTS

The discriminant function coefficients of the nine parameters are presented in Table 43. For arithmetical ease all coefficients were multiplied by 10³ and rounded to the nearest whole number.

The score was obtained by addition of the existing parameters in each patient. Fig. 8 shows the histograms of these discriminant scores of the patients in the two groups.

Patients who died during the hospital stay are shaded in Fig. 8 in order to show that this diagnostic index does not represent a prognostic index.

Specificity at different levels of sensitivity was calculated according to the formulae presented in part II (page 1) and graphically illustrated in Fig. 9.

At an index score below -5 the sensitivity was 100 per cent with a specificity of 16 per cent.

DISCUSSION

As has been pointed out in part II of the present study a discriminant function analysis of the kind performed in the present study ought to be seen as an attempt to identify symptoms and signs which are essential for the description of a disease and more important for differentiation between diseases.

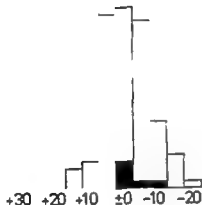
per cent
30-

□ AMI-patients
▨ ICS-patients



per cent
30-

□ OBS-patients



■ Dead during hospital stay

Fig 8 Distribution of the clinical diagnostic index in the AMI- and ICS-patients compared to that of the OBS-patients. The scores are derived from the discriminant function analysis. (Black areas represent patients dead during the hospital stay)

Bruce and Yarnall (1966) stated that in the physician-computer interaction the computer will not relieve the physician of his responsibility of making decisions regarding each specific patient. The effect of this interaction is, according to these authors, the motivation of the physician to attempt to be more rigorous in defining concepts, in collecting information, and in making reasonable decisions.

"Next there is the fact that what is easy for the computer may be difficult for men, and what is easy for men may be extremely difficult for the computer (Sterling and Pollack, 1965)

Cohn and Gorlin (1972) stressed that this type of indices does not represent a diagnostic panacea, but it does give a diagnostic guideline.

In the present study it has been shown that a history of smoking and a history of unstable angina defined according to the Edinburgh group (Fulton et al., 1971) are parameters which increase the probability of an AMI in a patient with an attack of chest pain lasting more than one hour and with radiation of pain to the arms and/or extension of pain to the jaw. A pulse pressure below 60 mm Hg on admission also increases the probability of an AMI. If the patient has had

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Supplementum 544

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Special Reference to Obesity and
Fatty Acid Synthesis *de Novo*

Lars Sjöström

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SUPPLEMENTUM 544

FROM THE DEPARTMENT OF MEDICINE I, SÄRLÖFEN'S HOSPITAL,
UNIVERSITY OF GÖTEBORG, GÖTEBORG SWEDEN

ADULT HUMAN ADIPOSE TISSUE CELLULARITY AND METABOLISM

with Special Reference to Obesity and
Fatty Acid Synthesis de Novo

BY

LARS SJÖSTRÖM

GÖTEBORG 1972

This thesis is based on the following papers:

- I Sjöström, L Björntorp P & Vrana J : Microscopic fat cell size measurements on frozen-out adipose tissue in comparison with automatic determinations of osmium-fixed fat cells
J Lipid Res 12:521 1971
- II Sjöström L Björntorp P & Känsson J E : An optimal assay system for subcellular determination of de novo fatty acid synthesis in human adipose tissue
- III Björntorp P Bengtsson E Blohmé G Jonsson A Sjöström L Tibblin E Tibblin G & Wilhelmsson, L : Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women
Metabolism 20:927 1971
- IV Sjöström L Smith U Krotkiewski M. & Björntorp P : Cellularity in different regions of adipose tissue in young men and women Metabolism in print Dec 1972
- V Björntorp P & Sjöström L : Number and size of adipose tissue fat cells in relation to metabolism in human obesity
Metabolism 20:703 1971
- VI Sjöström L : Fatty acid synthesis de novo in human adipose tissue I Effects of a high-carbohydrate diet in obesity of different cellular types
- VII Sjöström L : Fatty acid synthesis de novo in human adipose tissue II Effects of a high-carbohydrate diet combined with a positive energy balance in obesity

These papers will be referred to by their Roman numerals

The English was corrected by John Kral M A M D

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INTRODUCTION

The adipose tissue mass of the body depends on the size and total number of the fat cells. This simple fact has been known for a long time (Cf 91) but in the absence of adequate techniques adipose tissue cellularity has not been systematically studied until recently. Pioneer studies were made by Reh (91) and Bjurulf (5) who studied fat cell sizes in different regions of the body. The clinical implications of their studies were limited since the studies were solely made on material from autopsies preventing among other things relating the cellularity of adipose tissue to metabolism. The relationship between coronary arteriosclerosis and fat cell size in Bjurulf's work was however a most important finding which indicated the necessity of further work in this field.

To study these problems an adequate method for determination of adipose tissue cellularity in vivo was obviously required. The percutaneous adipose tissue needle biopsy technique of Hirsch et al (58-60) proved to be a very useful method to obtain adipose tissue specimens in a non-traumatic way which is necessary in research in the clinic. Since the available techniques for determination of fat cell size could not be used on needle biopsies new methods were worked out (for review see I). An excellent but expensive osmium fixation technique was described by Hirsch and Callian in 1968 (59). At the same time that this method was published a simple microscopic technique for determination of fat cell size was in use in this laboratory. Comparisons of the two techniques demonstrated a close agreement (I).

Accurate estimations of body fat were made possible through the development of under water weighing and isotope dilution techniques (for review see 70-83). Body fat divided by the mean fat cell size obtained from determinations in one or several parts of the body gave an estimate of the total number of fat cells in the body. Studies on adipose tissue cellularity according to these principles were first published by Hirsch, Knittle and Salans (62). Later more detailed information has appeared on the total cellularity (III-IV-V)

14 16 24 28 30 33 95) and the local cellularity (IV) of adipose tissue

Obviously the function of any tissue is determined by the cells and not inert substances as for instance calcium salts in bone. In adipose tissue this is of particular interest because the inert lipid droplet is located within the fat cell and constitutes most of the volume of the cell and the tissue as a whole. In contrast the metabolically active cytoplasm of the adult fat cell only constitutes a small part of the total volume of the cell.

The concept of the fat cell as the functional unit and reference basis in in vitro experiments on carbohydrate and lipid metabolism proved to be of considerable value. The first such experiments were published in the mid 60-ies by Björntorp et al (8 9 17 63) and Hirsch Knittle and Salans (62 96 97). Later correlations between adipose tissue cellularity and metabolic variables in vivo were reported from this laboratory. The most important finding seemed to be the positive correlation between fat cell size and plasma insulin concentration after an over-night fast and/or during a glucose tolerance test found in randomly selected middle-aged men (III 12 16) in obese patients (V) and in patients with endogenous hypertriglyceridemia (15). In obesity (113) and in 19 not specified cases (114) these observations have later been confirmed by other groups.

Insulin correlated less strongly to body fat than to fat cell size (V 16) and this interesting observations called for an explanation. It is a well-known fact that obesity is associated with a high incidence of decreased glucose tolerance (27 67 87) in spite of abnormally elevated insulin levels (3 27 68 88 97). Several investigations indicate that in vitro large fat cells are less sensitive to insulin than small ones (47 64 94 97 111). One possible explanation for this might be that the hyperinsulinemia of obesity is a compensatory adaptation to a peripheral insulin resistance in adipose tissue as first suggested by Salans Knittle and Hirsch (97). For such an explanation to be valid the total uptake of glucose by adipose tissue should be of such a magnitude that resistance to this uptake would cause an increase of blood glucose resulting in a

concomitant increase of insulin secretion. The present work (II VI VII) is a part of the effort made in this laboratory to determine the extent of glucose uptake of human adipose tissue in vitro and in vivo (9 10 11 19)

Since fatty acids are the most concentrated form of energy known in nature a large synthesis de novo of these acids in adipose tissue would be expected to be an important means of storing glucose carbon. In several species this is in fact the case (74 86). Since the adipose tissue mass in man is comparatively large it is of interest to investigate the quantitative importance of the fatty acids synthesized de novo in human adipose tissue as possible acceptors of glucose carbon. This seems to be of particular interest in conditions with peripheral insulin resistance as discussed above.

Because of large discrepancies in available results (for review see II) it was difficult to evaluate the quantitative importance of de novo fatty acid synthesis in human adipose tissue. Some authors reported that fatty acid synthesis could reach levels of quantitative importance (29 49) while others reported that necessary enzymes for fatty acid synthesis were virtually absent in human adipose tissue (103-106). It is plausible that these deviating results depended on differences in feeding states, types of subjects or assay techniques. With whole cell techniques dilution of labelled precursors might conceal a significant amount of de novo synthesized fatty acids. Subcellular techniques on the other hand usually do not reflect in vivo rates of fatty acid synthesis since among other things such a limiting factor as substrate availability is circumvented.

Since most reports on human adipose tissue indicated that fatty acid synthesis de novo in fact is low it was considered of interest to ascertain the maximum overall capacity of the involved enzymes and thus the upper limit of de novo fatty acid synthesis. Preliminary experiments in this laboratory indicated that available spectrophotometric assays of one of the individual enzymes in the sequence of fatty acid synthesis were not applicable in human adipose tissue studies and that the crude cytoplasmic assay systems so far used (66 103-106) were not optimal. Therefore an assay system (II) was worked

out that determined the incorporation of labelled acetyl units into fatty acids at a rate approaching the maximum overall capacity of the enzymes for the synthesis of fatty acids. This assay system was used in experiments with different diets (VI VII) in an attempt to estimate an upper limit for fatty acid synthesis.

To sum up the aim of the present investigation was to:

- 1 develop an adequate method for determination of fat cell size in man
- 2 investigate adipose tissue cellularity in obese subjects and controls and to search for correlations in vivo between cellularity and metabolism
- 3 develop an optimal subcellular assay system to determine the maximum capacity of the enzymes for fatty acid synthesis de novo in human adipose tissue and thus estimate the maximum capacity for this synthesis

METHODS

Fat Cell Size

Techniques for determination of fat cell size are detailed in paper I. A short summary is given here and some additional information is given in an Appendix.

Microscopic techniques A small adipose tissue sample (2 - 15 mg) was fixed in 35 % formaldehyde for 7 minutes. The sample was then frozen on the table of a microtome by carbon dioxide snow (1). Lately a tissue freezer (Kryofix H and M, WKF Brandau/Darmstadt West Germany) has been used instead to freeze both the adipose tissue sample (Kryofix H) and the knife (Kryofix M). The new procedure does not influence the quality of the slices and is easier and less expensive to use.

A frozen-cut slice 200 μ m in thickness was microscopically examined in a closed glass chamber containing Ringer's solution. A scale divides the eyepiece of the microscope in 100 parts. One scale unit corresponds to 2.44 μ m. The mean fat cell weight from 100 measured diameters could be calculated in two different ways (Methods I:a and I:b) as described in paper I and Appendix. In Method I:a the mean fat cell volume was an average of 100 volumes calculated on the separate diameter determinations. In Method I:b the mean fat cell volume was calculated from the mean diameter and its standard deviation according to the formula by Goldrick (55). Weights were calculated from volumes using the density of human fat cell triglycerides (70) in both methods. For routine purposes Method I:b was used.

Osmium fixation techniques With these techniques osmium fixed fat cells suspended in an electrolyte were measured and/or counted with an electronic particle counter. A Coulter Counter Model B as well as a Celloscope 302 was employed. A Celloscope 302 with a 600 μ m capillary tube was used in the final comparisons between the

osmium fixation method and the microscopic method. Suspensions of osmium fixed fat cells were prepared according to Hirsch and Gallian (59) with the improvements given in paper I. The fat cell size was determined according to Methods II:a or II:b described in paper I and Appendix. The calculation procedure of Method II:a was identical to that of method III of Hirsch and Gallian (59). Principally the total lipid weight of a sample is divided by the number of fat cells in that sample. Method II:b was a new procedure to determine the mean fat cell volume by measuring with the counter. Determinations of the sample wet weight and the ratio of lipid to wet weight were circumvented which is an advantage when only small adipose tissue samples are available.

Body Composition

Direct measurements. Body height was measured with the subject standing. Body weight without clothes was recorded on a scale with a sensitivity of ± 0.1 kg. Circumferences were measured with the subject standing. In men the maximum waist circumference was recorded during expiration. In women the largest gluteal circumference was recorded. In order to achieve highest possible reproducibility all measurements were performed by the same person. Triceps, subscapular and abdominal skinfolds were measured with a Harpenden caliper (45).

Body potassium was determined either by isotope dilution technique using ^{42}K (exchangeable potassium) or with a whole body counter (total body potassium ^{40}K). Exchangeable potassium was calculated from the specific activity of $^{42}\text{K}/^{39}\text{K}$ in the urine after 36 hours equilibration of 100 μCi of ^{42}K taken per os. This procedure has been described in detail earlier by Lindholm (78) and by Berg and Isaksson (4). Total body potassium was determined in a whole body counter (109)*. The whole body counter was calibrated by measuring the counts from subjects before and after administration of known

* Performed by B. Arvidsson, Ph.D., at the Radiation Physics Department, Sahlgren's Hospital, Göteborg, Sweden.

amounts of ^{42}K (2) Total body water was essentially determined as described by Lindholm (79) and by Berg and Isaksson (4) Tritium (400 μCi) taken per os was equilibrated for 5 hours The activity of a vacuum distillate of urine from the 6th + 7th hours and 8th + 9th hours was determined and the mean activity of the two samples was used to calculate total body water Close agreement between the activity of the two samples indicated complete equilibration

Calculated body compartments In randomly selected middle-aged men and women (III) body fat was determined anthropometrically The regression equations given below were found to be most accurate up to 30 kg of body fat when comparing with body fat determined with the isotope technique (see below) in other samples of the same population

Men (16):

$$\text{BF} = 0.381 \text{ CW} + 0.019 \text{ SF} - 24.78$$

(BF is body fat in kg CW waist circumference in cm and SF sum of the three skinfold measurements in mm)

Women (III):

$$\text{BF} = 0.65 \text{ Cg} - 47.5$$

(Cg is the gluteal circumference in cm)

In papers IV-VII body fat body cell mass and extracellular water were calculated from the isotope measurements as described by Berg and Isaksson (4)

Adipose Tissue Cellularity

The total fat cell number of the body was calculated by dividing body fat with an estimated average body fat cell weight* calculated from one or several regions

* In this thesis mean fat cell weight indicates the value calculated from 100 fat cell diameters in a slice from one adipose tissue site Average body fat cell weight refers to the estimated mean fat cell weight of the whole body and is obtained as an average of available mean fat cell weights

The local fat cell number was evaluated with a new approach using regional measurements of mean fat cell volume and skin plus adipose tissue thickness. The mean fat cell volume was determined with the microscopic technique (I) and the skin plus adipose tissue thickness with a modification (see IV) of the ultrasonic technique described by Booth et al (25).

The calculation of local fat cell number was based on a cylinder with a base surface of 1 mm^2 and a height equal to the skin plus adipose tissue thickness. The assumption was made that this thickness was solely made up of adipose tissue since it was not possible to discriminate between the echo originating from the dermis-adipose tissue border and the initial pulse.

The "extracellular" volume in the cylinder was found in Bjurulf's diagram relating extracellular space (=non fat cell space) to cell size (6), at the appropriate mean fat cell size. The total intracellular fat cell volume of the cylinder was obtained as the difference between the total volume and the extracellular space of the cylinder. Local fat cell number was then calculated by dividing the total intracellular fat cell volume of the cylinder with the mean fat cell volume of the region.

Blood and Urine Chemistry

When not stated otherwise all analyses of blood were performed on venous blood samples taken after an over-night fast. The adipose tissue biopsies were taken after the blood samples.

After extraction according to Polch (50) serum triglycerides were determined as described by Carlson (34) and serum total cholesterol by the method of Cramér and Isaksson (37). Blood glucose was determined by the glucose oxidase method of Keston (69) as modified by Levin and Linde (75). The Glox[®] assay kit (Kabi AB Stockholm Sweden) was used. Plasma insulin was determined with the radio-immunological method of Hales and Randle (57) using assay kits from Amersham in papers III-V (Insulin Immunoassay kit The Radiochemical Centre Amersham Buckinghamshire England) and from

Pharmacia in papers VI and VII (Phadebas^R Pharmacia Uppsala Sweden) Urine nitrogen was determined at the Institute of Clinical Nutrition Göteborg Sweden A micro Kjeldahl method was used utilising a Technicon autoanalyzer with digester model 1 (Technicon Controls INC Chauncey New York USA)

Adipose Tissue Metabolism

Whole cell technique The incorporation of glucose into CO_2 triglycerides and fatty acids by adipose tissue slices was studied with a conventional technique (VI) Two 200 mg specimens of adipose tissue were incubated in duplicate in 3 ml Krebs-Ringer-Bicarbonate solution (115) (half the prescribed concentration of Ca^{2+}) The gas phase was $\text{O}_2:\text{CO}_2/95:5$ The water phase contained 5.5 mM glucose and $15 \cdot 10^6$ cpm [$\text{U-}^{14}\text{O}$]glucose When indicated 1000 μU insulin per ml was added

Subcellular technique Fatty acid synthesis de novo was determined in an optimal fortified cytoplasmic assay system (II) measuring the incorporation of acetyl units from acetate and citrate at a rate which probably approached the overall maximum capacity of the present enzymes (VI VII)

Potter-Elvehjem homogenates (90) of adipose tissue were subjected to high-speed centrifugation yielding a fat fraction a soluble cytoplasmic fraction and a sediment of nuclei mitochondria and microsomes Practically all lipogenetic activity was found in the soluble cytoplasmic fraction of the homogenate This activity represented a de novo fatty acid synthesis as demonstrated by TLC avidin inhibition and CO_2 -dependence The main products were myristic and palmitic acids

Fig 1 demonstrates the principle of the assay system. Curve I represents C^{14} -acetate (2.5 mM) incorporation at different unlabelled citrate concentrations Curve II shows the incorporation of acetyl units from C^{14} -citrate in the presence of 2.5 mM unlabelled acetate Except for the labelling curve I and curve II are identical Thus,

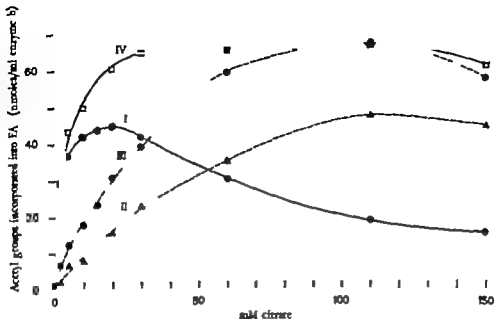


Figure 1 Influence of citrate concentration on $[1-C^{14}]$ acetate and $[1-C^{14} \ 5-C^{14}]$ citrate incorporation into fatty acids. For explanations see text.

the sum of curves I and II (=curve IV) shows the total incorporation of acetyl units derived from acetate and citrate at different citrate concentrations. Curve III represents C^{14} -citrate incorporation at different citrate concentrations without acetate present in the assay system. The results in Fig. 1 suggested that at least three different types of incorporation might give equal incorporations of acetyl units viz C^{14} -acetate plus C^{14} -citrate incorporation with 30 mM citrate, C^{14} -acetate plus C^{14} -citrate incorporation with 110 mM citrate and finally 110 mM C^{14} -citrate incorporation without acetate present in the assay system. A number of experiments shown in Table 1 however demonstrated that this unfortunately was not the case. Although the three types of incorporation gave results of the same magnitude for a given enzyme preparation in individual experiments these results sometimes differed more than could be explained by the error of the method. From the experiments in Table 1 it seemed

Tabl 1 The influence of citrate concentration on acetat and citrat incorporati n i to fatty acids

The Roman numerals of the columns correspond to curve numbers in Fig 1 with respect to assay conditions

Exp no	Citrate conc (mM)	Incorporati n of acetyl groups from			Sum of acetate (I) and citrate (II) incorporation
		acetat (I)	itrate (acetat present) (II)	itrate (acetate excluded) (III)	
			nmol s/ml hour		(IV)
1	30	57 1	30 9	87 7	88 0
	110	39 1	67 2	120 9	106 3
2	30	35 2	19 9	48 8	55 1
	110	24 9	26 0	63 4	50 9
3	30	3 4	3 2	4 0	8 6
	110	3 1	3 9	7 2	7 0
4	30	10 1	4 8	8 3	14 9
	110	3 7	12 6	13 8	16 3
5	30	12 6	4 8	15 1	17 4
	110	8 8	17 7	30 4	26 5
6	30	88 6	109 6	93 5	198 2
	110	54 1	128 1	180 4	182 2
7	30	48 5	67 8	55 9	116 3
	110	28 1	72 2	104 3	100 3
8	30	56 2	62 4	122 4	118 6
	110	24 1	61 2	130 1	85 3
9	30	43 3	33 3	50 0	78 6
	110	18 1	44 2	89 1	62 3
10	30	44 2	36 2	47 1	80 4
	110	35 0	51 2	53 9	86 8
Mean [±] SD	30	40 1 [±] 33 6	37 3-34 0	53 2-38 8	77 6 [±] 38 4
	110	33 9-16 3	48 4-36 8	79 4 [±] 35 8	72 3 [±] 32 1

Analysis f variance

all ight group F(7 72) 2 02 n

only the three groups with mean valns in box F(2 27)=0 04 n s

possible to conclude that determinations of acetate plus citrate lipogenesis at both 30 and 110 mM citrate revealed the maximum or almost maximum incorporation of acetyl units in most individual cases These incorporations have been used throughout the present investigation Incorporation of 110 mM C¹⁴-citrate without acetate present was a fairly good approximation of the maximum capacity but was occasionally much lower

Statistical Methods

The statistical methods are presented in the separate papers (I-VII). Ordinary group comparison t-test, pairing design t-test, linear regression and analysis of variance have been used. Significant P-values have been further analysed with tests according to Scheffe (99), Dunnett (Cf 77) or Tukey (Cf 77). In paper VI a balanced block design of analysis of variance was carried out by an IBM-computer according to the program BMD 02V in Analysis of variance for factorial design (43).

Error of Methods

The standard error of a single determination (38) expressed in % of the mean of 40 - 100 double determinations for different analysis are presented below: Glucose 2.5 %; Insulin (Amersham) 7.1 %; Insulin (Pharmacia) 4.1 %; Triglycerides 2.5 %; ^{42}K 4.5 % (ref 78); total body water 4 %; fat cell diameter \pm 5 %; fat cell weight 8 %; acetyl unit incorporation by cytoplasmic assay 3.0 - 14 % for different types of incorporation see paper II.

As repeatedly noted in the literature (22, 97) the error of methods are large for metabolic human whole cell experiments. In the present investigation the errors were: Glucose incorporation into CO_2 27 %; glucose incorporation into triglycerides 20 %; glucose incorporation into fatty acids 24 %. The errors calculated on incorporation values expressed per g TG.

Fifteen consecutive determinations of serum cholesterol were performed with regular intervals during 6 months on a control serum (Hyland, Costa Mesa, Calif, USA). The mean value of these determinations was 174.1 ± 6 mg, corresponding to a coefficient of variation = 3.4 %. The coefficient of variation for ^{40}K was 1.7 % (ref 2).

In the present investigation all analyses except cholesterol and ^{40}K were performed in duplicate.

DISCUSSION OF THE METHODS

Comparison of Different Fat Cell Sizing Methods

When measuring a large number of fat cell diameters a normal distribution is obtained (Cf Fig 5 in paper I) Mean fat cell weight determinations according to Methods I:a and I:b gave almost identical results (Fig 2) The formula for mean fat cell weight determination used in Method I:b is valid only when the cell diameters are normally distributed Thus the strong correlation between results obtained with the two methods demonstrates that counting 100 cell diameters resulted in a distribution sufficiently close to the normal distribution For routine measurements (II-VII) Method I:b has been used

Results with Method II:a and Method II:b correlated closely both when measuring with the Celloscope ($r=0.998$) and the Coulter Counter ($r=0.954$) (Fig 7 in paper I)

Since the microscopic methods may be criticised because of the

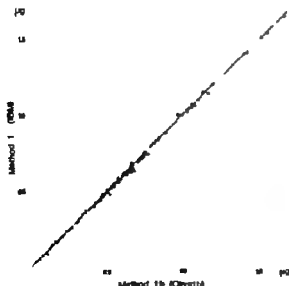


Figure 2 Comparison of the two calculation procedures of the microscopic method for determination of fat cell size $y = 0.993x - 0.001$
 $r = 0.999$ $p(t_p) < 0.001$ $n = 273$

small number of counted cells and the risk for subjective errors it was necessary to compare the microscopic method with an objective one. The osmium fixation techniques were considered to be the most objective ones so far available since large numbers of cells are measured electronically. However this does not exclude important subjective errors in the osmium technique since the subjectively determined inflection point of the cumulative curve corresponding to the size of the smallest fat cells in the sample is often indistinct. The error of the osmium technique has not been reported by Hirsch and Gallian (59). According to Brook (32) the coefficient of variation of method III of Hirsch and Gallian (=Method II:a in paper I) is 7.5 %. This error is of the same order as the error of the mean weight determinations with the microscopic method (8 % paper I).

Even if it is not possible to establish which method is the most objective one there was a close correlation between fat cell weights determined with the microscopic Method I:b and the osmium fixation Method II:a ($r=0.960$) and the regression line did not differ significantly from the identity line (I). In comparison with the osmium techniques the microscopic method is simpler, speedier and much cheaper.

Choice of System and Substrate for the Study of Fatty Acid Synthesis de Novo

As was mentioned in the introduction most previous investigations seemed to indicate that fatty acid synthesis de novo in human adipose tissue is of little quantitative importance. In order to estimate the maximum capacity of the enzymes for this synthesis in different physiological and pathological conditions an optimal cytoplasmic assay system was developed. Mitochondria and microsomes were excluded from the system to avoid β -oxidation as well as chain elongation of fatty acids. A crude cytoplasmic system was chosen because the purification of individual enzymes would result in a loss of activity which would be difficult to control thus making quantitative studies uncertain. The activity for the synthesis of fatty acids

was almost completely recovered in the soluble fraction of the adipose tissue homogenate (66 Table 1 in paper II) This also seems to be the case in fractionated homogenates of rat adipose tissue (92)

Different radioactive precursors were considered of varying validity as substrates in assay systems set up to measure the maximum capacity of the enzymes for fatty acid synthesis Because of this the following considerations were taken into account:

Labelled acetyl-CoA has often been used as a precursor in sub-cellular studies of lipogenesis in different species and organs (53 54 82 85 105 106 118) Acetyl-CoA incorporation into fatty acids however requires the presence of some tricarboxylic acid since these acids activate acetyl-CoA carboxylase [E C 6 4 1 2] (for review see 116) The most potent activator is citric acid (82) Citrate however is also an important precursor in acetyl-CoA formation via ATP citrate lyase [E C 4 1 3 8] (for review see 52) If measures are not taken to prevent acetyl-CoA formation from citrate and CoA (obtained by decarboxylation of labelled acetyl-CoA or malonyl-CoA) an uncontrolled dilution of labelled acetyl-CoA may result making quantitative determinations impossible

Malonyl-CoA is the product of the acetyl-CoA carboxylase reaction This step is commonly considered rate-limiting in different species and tissues (26 36 53 65 82 85 110 118) and thus malonyl-CoA is not an appropriate precursor to study the limitations of the enzymes for fatty acid synthesis The activity of acetyl-CoA carboxylase has been determined by measuring $^{14}\text{CO}_2$ -fixation from $\text{H}^{14}\text{CO}_3^-$ (82 106) Labelled CO_2 is presumed to be fixed to malonyl-CoA This seems to be an adequate assumption when working with purified acetyl-CoA carboxylase (82) but not with crude cytoplasmic systems (106) since other carboxylase reactions might occur (119) Furthermore contrary to most earlier reports recent experiments indicate that fatty acid synthetase may have a lower activity than acetyl-CoA carboxylase in adipose tissue in the rat (35) For these reasons measurements of the CO_2 -fixation from $\text{H}^{14}\text{CO}_3^-$ was not considered adequate for the present purpose

Since pyruvate dehydrogenase [E C 1 2 4 1] is an intramito-

chondrial enzyme (98) pyruvate can not be used as precursor in cytoplasmic systems For the same reason none of the glycolytic intermediates preceding pyruvate can be utilized though they are metabolized in the cytoplasmic compartment (40)

Acetyl(-)carnitine probably is of minor importance as an acetyl-CoA precursor in fatty acid synthesis (31)

The remaining precursors of extra-mitochondrial formation of acetyl-CoA and fatty acids seem to be acetate and citrate The present assay system measured the simultaneous incorporation of acetyl units from these precursors at a rate probably approaching the maximum overall capacity of the present enzymes to synthesize fatty acids de novo In comparison with systems using acetyl-CoA as a precursor the assay system described here depends on two additional enzymes that can possibly be rate-limiting: acetyl-CoA synthetase [E C 6 2 1 1] and ATP citrate lyase

RESULTS

Adipose Tissue Cellularity

Body fat average body fat cell sizes and total fat cell numbers in investigations III IV and V are summarized in Table 2 In the table the results in paper V have been divided according to sex In Table 3 results are given from consecutive measurements of fat cell weights in three adipose tissue regions The subjects in Table 3 ranged from slim to severely obese Close correlations were demonstrated between fat cell sizes in the different regions in both men and women

Control subjects In randomly selected middle aged men (n=49) and women (n=58) body fat was larger in women than men but the difference could not be explained in terms of average body fat cell weight or total fat cell number If sick subjects and those on a diet were excluded (Table 1 in paper III) fat cell weight as well as fat cell number correlated positively with body fat in both men and women These correlations were also found in 11 male students (22 - 24 years) (III)

The body weight of middle-aged men at about 20 years of age could be traced from military records Middle-aged men who had increased more than 10 kg in body weight during the last 35 years were compared to those who had kept a steady weight and to the male students The men who increased in weight had more body fat than the other two groups caused by an increased average body fat cell weight There were no differences in fat cell number between the three groups Middle-aged men with a steady weight and male students had similar fat cell weights

In an attempt to explain the well-known differences between the sexes with respect to body fat and subcutaneous adipose tissue thickness (81 117) adipose tissue cellularity was determined in young male (n=11) and female (n=13) students who had a weight-index close to 1.0 (IV) From determinations of total as well as local

Table 3 Mean fat cell weight and intercorrelations in three adipose tissues in 75 consecutively examined men and women.

	Men		Women	
Number of subject	75		75	
Fat cell weight μg^2				
range all regions	0.07	1.57	0.11	1.74
Xyogastric (mean \pm SD)	0.46	± 0.26	0.64	± 0.34
Femoral (mean \pm SD)	0.53	± 0.27	0.75	± 0.37
Gluteal (mean \pm SD)	0.51	± 0.26	0.75	± 0.32
$r(2/222)$	1.41		2.56	
p			<0.10	
<u>Correl tissue</u>	Exv	r	Exv	
X Y				
Xyog femoral	$y=0.91 \pm 0.10$	0.87***	$y=0.96 \pm 0.13$	0.89***
Xyog vs gluteal	$y=0.88 \pm 0.10$	0.88***	$y=0.79 \pm 0.24$	0.84***
Femoral vs gluteal	$y=0.83 \pm 0.07$	0.86***	$y=0.79 \pm 0.16$	0.91***

*** $p < 0.001$

fat cell numbers it was demonstrated that the greater body fat of women was mainly caused by an increased fat cell number. The average body fat cell weight and the distribution patterns of adipose tissue thickness and local fat cell number were not significantly different between the sexes (IV).

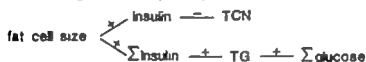
The middle-aged men and women (III) had more body fat than the male and female students (IV) respectively. This was explained by larger fat cell sizes in both the middle-aged populations while there were no differences in total fat cell numbers between the groups (IV).

Obese subjects The body fat of the obese subjects ($n=37$) (V) was about 3 times greater than in randomly selected middle aged controls (III). There was a strong positive correlation between body fat and total fat cell number. Contrary to the control groups body fat and fat cell weight did not correlate in the obese groups. There were no differences in body fat or adipose tissue cellularity between obese men and obese women (Table 1). On an average the obese subjects had 72 % larger fat cell weights and 52 % more fat cells than

the controls. In moderate obesity (25 - 40 kg body fat) increased cell size determined the greater quantity of body fat compared to controls. It was suggested that these obese subjects might be separated as a clinical subunit designated hypertrophic obesity. With increasing obesity an increasing number of fat cells contributed proportionately more to body fat (V). Generally this hyperplastic obesity was combined with large fat cells. Pure hyperplastic obesity appeared to be found only in subjects who were sick or on a reducing diet (Of Table 1 in paper III)

In vivo correlations The significant correlations between adipose tissue cellularity and metabolism reported in papers III, IV and V are summarized in Fig. 3. In obese subjects and in middle-aged men fat cell size correlated positively with fasting insulin and with the sum of insulin values during the oral glucose tolerance test.

Middle-aged men (n=49):



Middle-aged women (n=23):

no correlations

Obese subjects (n=26):

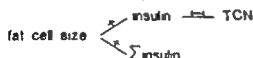


Figure 3 Correlations between adipose tissue cellularity and metabolism reported in papers III-V. Correlations are indicated with + and -. Signs within brackets indicate trends toward significant correlations ($p < 0.10$).

These correlations were not found in middle-aged women. In middle-aged men the sum of the insulin values correlated with serum triglycerides which in turn correlated with the sum of glucose values during the oral glucose tolerance test. Almost identical correlation coefficients were obtained when the fat cell size was expressed as diameter, surface or volume (not shown in Fig. or Tables).

Adipose Tissue Metabolism

Precursors of fatty acid synthesis *de novo*. Contrary to earlier reports by Shrago et al (103-106) the present investigations (II VI VII) have indicated the presence of ATP citrate lyase [E C 4 1 3 8] in human adipose tissue. In vitro the capacity of the enzymes for citrate incorporation is at least as large as the capacity for acetate incorporation into fatty acids if optimal conditions are used for both precursors (II VI VII Table 1).

Fatty acid synthesis in adipose tissue in different regions of the body. To find out if the fatty acid synthesis in any single region is representative of the adipose tissue as a whole the synthesis of fatty acids was determined in five different regions using the subcellular system. Details of the experiments and results are presented in Fig. 4.

Expressed per g TG the activity of biopsies taken toward the end of the operation (E) was about 15 % lower than biopsies in the same region from the beginning of the operation (e) ($e = 21.8 \pm 18.0$ $E = 18.2 \pm 15.8$ nmoles/g TG mean \pm SD $n=4$ $p < 0.05$ paired comparisons). For this reason the "E" values were excluded from further calculations. Fig. 4 clearly demonstrates that variations within the subjects were much less pronounced than between different subjects. There were significant differences between the mean values of all the subjects except the two with the lowest levels of activity (Fig. 4 top section). On the other hand no differences between the different regions could be demonstrated when values from the six patients were pooled. In fact the variation between different

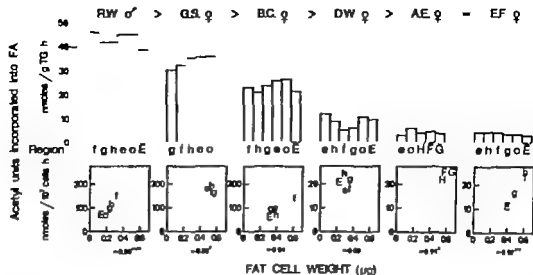


Figure 4 Enzyme activity for fatty acid synthesis in different adipose tissues. The adipose tissue biopsies from the femoral (f), gluteal (g), hypogastric (b), bicipital (p) and omental (o) regions were performed in five women and one man in connection with cholecystectomy. Except for their gallbladder disease the patients were generally healthy. They were premedicated with diazepam and tropic. The biopsies in each subject were taken in the same order as the symbols are arranged. Small letters signify that the biopsy was removed within 30 minutes of the induction of the narcosis with hexobarbital oxygen nitrous oxide and succinylcholine. After these biopsies were taken the patient was sedated with flunitrazepam. When indicated, subcutaneous biopsies were taken at the end of the operation (b, k, l letters) after an average of 90-120 minutes from the incision.

Incorporation of acetyl unit from $[1-^{14}C]$ acetate and $[1-^{14}C]$ 3- $[^{14}C]$ citrate was determined with the cytoplasmic assay system using 30 mM citrate (Cf. "Methods"). Activity expressed per g TG (top) or per 10^7 fat cells (bottom section). The results in the top section were examined with analysis of variance according to two-way classification with single observation in each cell (76). The F-values were excluded from calculation. There were highly significant differences between subjects $[F(5, 20) 385, p < 0.001]$ but no difference between different sites $[F(4, 20) 2.01]$. The residual standard deviation was 1.4 units which is equal to 6.9% of the mean.

Product moment correlations (r) between fat cell weight and activity per cell are given at the bottom of the figure calculated on the first biopsies. $p < 0.10$; $p < 0.02$; $p < 0.01$; $p < 0.001$. If F-values are included in the calculations equal or higher degrees of significance are obtained.

regions was not larger than the error of the method.

Expressed per 10^7 fat cells there were large variations in fatty acid synthesis between different regions. However, these variations were explained by different cell sizes in different regions. A positive correlation between fat cell weight and activity per cell could be demonstrated (Fig. 4 bottom section).

In five subjects (RW excluded) regional studies of glucose incorporation into fatty acids in slices were also performed. Similar

results were obtained but the intra-individual variations were much larger than in the subcellular assay making the statistical analysis less convincing (not shown in Fig)

Effects of adipose tissue cellularity In the whole cell or subcellular systems there were no significant differences in fatty acid synthesis expressed per g TG between normal hypertrophic hyperplastic or combined hyperplastic-hypertrophic adipose tissue (VI)

Expressed per cell large fat cells showed a trend toward greater whole cell fatty acid synthesis In the cytoplasmic assay system this tendency reached full statistical significance (VI) These findings on whole cell and subcellular fatty acid synthesis in relation to cell size have previously been demonstrated also in fat cells separated into different size classes from the same adipose tissue (20)

Rate-limiting factors in fatty acid synthesis de novo As discussed in papers VI and VII the high ratio of cytoplasmic acetyl unit incorporation into fatty acids/whole cell glucose incorporation into fatty acids calculated on a molar basis indicates a) that the supply of acetyl units and not the capacity of fatty acid synthesizing enzymes is rate-limiting in fatty acid synthesis and b) that the incorporation rate of acetyl units determined by the cytoplasmic assay system may approach the maximum capacity of the enzymes The latter point is supported by the fact that palmityl(+)carnitine does not significantly increase the incorporation of acetyl units into fatty acids (VII)

Using whole cell preparations incorporating glucose and acetate into fatty acids Del Boca and Platt (41) and Saggerson and Greenbaum (92) demonstrated that the supply of acetyl units is rate-limiting for fatty acid synthesis in rat adipose tissue (see VII) When paper VII was submitted for publication identical experiments had not been performed on human adipose tissue but an additional interpretation of human whole cell data presented by Björntorp et al (19) made it possible to arrive at the same conclusion (VII)

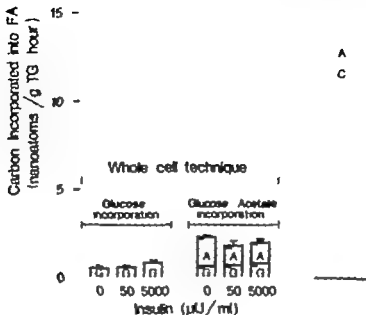


Figure 5 Rate limitation of fatty acid synthesis in whole cell technique Adipose tissue specimens from man undergoing cholecystectomy were incubated and analyzed as described in Methods. With 0 and 5000 μU insulin/ml triplicate were analyzed with 50 μU/ml duplicate. The left group of columns shows the incorporation of carbon from 5.5 nM glucose (G) (2×10^4 cps [14 C]glucose) with the insulin concentrations indicated. Bars represent 1 SD. In the right group of columns 5.5 nM glucose and 5.5 nM acetate were present in the medium. In one series of incubations glucose (G) was labelled (2×10^4 cps [14 C]glucose) and in another acetate (A) was labelled (8×10^4 cps [14 C]acetate). The bars with the column indicate the 1 SD interval for incorporation of carbon from glucose or acetate. On SD for the incorporation of carbon from acetate plus glucose is indicated at the top of the G A-columns. Glucose and acetate incorporations were paired at random.

Glucose incorporation was insulin sensitive [$F(2, 5) = 17.9$, $p < 0.01$] but not in the presence of acetate. The acetate incorporation was smaller with than without insulin [$F(2, 5) = 4.1$, $p < 0.05$]. Without insulin present there was a trend toward larger glucose incorporation with than without acetate present ($p < 0.10$ paired comparison). The incorporation of carbon from glucose plus acetate was larger than from glucose alone (for 1 cubit with 0 μU insulin/ml $p < 0.001$; for 50 μU/ml $p < 0.10$; for 5000 μU/ml $p < 0.01$; paired comparisons).

Critical value was. The maximal incorporation of acetyl carbon from acetate (A) plus rate (C) measured as described in Method.

Data from a single experiment with the same design as in the rat experiments (41-92) using human whole cell preparations are shown in Fig 5. These results indicate that the sequence of enzymes active in the synthesis of fatty acids can not be rate-limiting for the incorporation of carbon from glucose alone since the addition

of acetate to the whole cell system doubles the total incorporation of carbon. This incorporation however is also far below the enzyme capacity to incorporate acetyl carbon into fatty acids as shown by the cytoplasmic assay (Fig 5 right column). The experiment is in accordance with the high subcellular/whole cell ratios demonstrated throughout papers VI and VII.

Effects of high-carbohydrate feeding Effects of a high-carbohydrate-low-fat diet were studied during long term moderately negative (VI) and short term positive (VII) energy balances. The glucose incorporation into fatty acids by slices as well as the capacity of the enzymes for fatty acid synthesis increased substantially by both treatments. However the higher levels reached during this stimulation (80 nmoles/g TG/hour) were of little quantitative importance. Glucose incorporation into glyceride-glycerol and CO_2 in slices was not changed by hypocaloric* high-carbohydrate feeding (VI) while hypercaloric feeding increased the incorporation into glyceride-glycerol (VII).

Both of the high-carbohydrate treatments increased serum triglycerides and decreased serum cholesterol while blood glucose and glucose tolerance were unchanged (VI VII). Hypercaloric high-carbohydrate feeding increased plasma insulin (VII) while hypocaloric feeding did not (VI).

Correlations (VI) The correlations between the fat cell size and the enzyme capacity for fatty acid synthesis increased during the course of high-carbohydrate feeding. During but not before high-carbohydrate feeding there were several correlations between different whole cell and subcellular activities of adipose tissue. During the feeding there were also strong correlations between the amount of fatty acids synthesized in adipose tissue and the levels of serum triglycerides. The latter correlation was most likely not due to a direct dependence between the two variables.

* In this thesis the terms hypo- iso- and hypercaloric refer to the amount of calories required to maintain a steady weight.

Adipose Tissue Cellularity

Determinations The total fat cell number of the body was calculated by dividing body fat with an estimated average fat cell weight. Obviously this procedure is inexact.

In animal experiments it is comparatively easy to determine body fat by removing defined regions of adipose tissue or by analysing the triglyceride content of the whole animal. Body fat determinations in man are of course much more complicated since direct measurements are not possible. Instead body fat must be estimated from body density, body potassium, tritium space or anthropometric measurements using several assumptions about the composition of the body. These approaches can all be criticised (73) but close agreements between different methods (44, 51, 71, 84, 102, 120) indicate that several of them may be adequate. In the present investigation body fat was calculated as one part of a four compartment system using both potassium and tritium isotopes (4). This procedure may result in smaller errors than calculations based on only one isotope though it can not be proved because of the lack of direct methods for comparisons.

A method to determine body fat independent of all assumptions about body composition has recently been published (73). The method which is based on the uptake of highly fat-soluble inert gases may turn out to be excellent for calibration of other methods though it at present seems too complicated for most clinical investigations.

While body fat probably can be estimated accurately enough the determination of the average fat cell weight of the body is apparently more complicated. To find out if this is at all possible it is necessary to make an analysis of the variations of fat cell sizes within and between different regions of adipose tissue in the body.

The variation within one region is rather limited since there is a close correlation between double determinations of the mean fat cell size in different specimens from the same adipose tissue site.

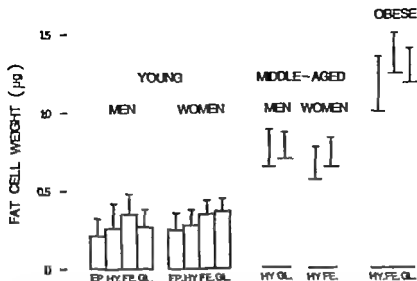


Figure 6 Mean fat cell sizes in different adipose tissue sites of subjects examined in paper III-V. The values for the middle-aged women refer to the mean fat cell weight in the hypogastric region of 23 randomly selected women and to the mean fat cell weight of the femoral region in 35 other randomly selected women. In middle-aged men (n=15) and obese subjects (n=23) mean values are calculated only on those subjects where complete data from the indicated sites were available.

($r=0.99$ paper I). The high correlation ($r=0.96$) between the microscopic (I:b) and the osmium fixation (II:a) method points in the same direction since Method I:b determines only 100 cells from one lobulus while Method II:a determines a large number of fat cells from several lobuli. Recently this type of evidence has been found when comparing results from Method I:b with microscopic measurements of collagenase liberated fat cells (112).

The variations in fat cell sizes between different regions have been examined in several types of subjects in the present work. The results are summarized in Fig. 6. There were no significant differences between the fat cell sizes of the epigastric, hypogastric, femoral and gluteal regions in young men (IV) or of the hypogastric and gluteal regions in middle-aged men (III) or of the hypogastric and femoral regions in middle-aged women (III). In young normal women (IV) and in obese subjects (V) the smallest mean fat cell weight

was 20 - 30 % smaller than the largest mean cell weight ($p < 0.05$) These differences would have been still larger if fat cells from adipose tissue in peripheral or intraabdominal regions had been investigated since fat cells in these regions are known to be smaller than in other regions (5 32 56 80 91 93 Cf Fig 4) Theoretically to determine the exact weight of the average fat cell of the body it would be necessary to know the total number of cells in each and every region of the body and the mean weight of the cells in each of these regions This is of course an impossibility However the differences in mean fat cell weight in different adipose tissue sites within the different groups of subjects mentioned above are small in comparison with the differences between the average body fat cell weight of these different groups (Table 2 Cf Fig 6) Furthermore there are significant correlations between the fat cell sizes of different sites within different groups of subjects (IV V 5) including correlations between subcutaneous omental and peripheral fat cells (5 56 80) Correlations between different regions in subjects with a wide range of fat cell sizes are very strong in both men and women (Table 3) All these facts indicate that it is appropriate to estimate an average body fat cell weight from mean fat cell weights from a few or with small intra-individual variations even one single adipose tissue site Thus it seems plausible that the total fat cell number of the body determined by dividing the body fat with the average body fat cell weight is an adequate index of the true number of fat cells in the body Since the smaller fat cells of peripheral and intraabdominal adipose tissue were not examined in the present investigations the average body fat cell weight is probably over-estimated and thus the total fat cell number under-estimated

Control subjects An analysis of adipose tissue cellularity in obesity or any other pathological condition requires a comparison with the so-called normal condition Though the distinction between obesity and normality is much disputed the problem usually is circumvented by choosing statistical definitions Disregarding such an obvious limitation of this approach as for instance the fact

that increased weight and obesity are not equivalent phenomena statistically defined borderlines are arbitrary Such arbitrary limits probably have to be accepted however until risk factors and diminished life-expectancy can be taken into account in the diagnosis of obesity

Since there is no generally accepted definition of normal weight and obesity it is not astonishing that the norms for adipose tissue cellularity have not been established In fact so far very little attention has been paid to the reference problem In table 4 results from the few papers providing data on both fat cell size and number have been summarized Investigations reporting only fat cell size are not included (47 48 56 72 80 93 97) The groups have been small (28 61 95) most of the controls have been chosen among the ill (28 33 61) and some of them have not been described with respect to age and sex (61) When age is presented the ranges have often been extremely wide (28 33) The collagenase technique used by Bray (28) can be suspected of selectively rupturing large fat cells since at a body fat of about 20 kg he obtains much smaller fat cells and thus a much higher total fat cell number than is reported in comparable studies (III 33 61) To sum up it seems that the cellularity of the adipose tissue of only 5 healthy subjects within a narrow age range has so far been reported in the literature (95) These subjects served as their own controls in an elegant investigation of experimental obesity performed by Salane Horton and Sims (95)

Obviously it might be hazardous to use the values obtained from these control subjects (28 33 61 95) as normal values when comparing with obesity Table 2 clearly demonstrates how different so-called normal materials might be even when examined by the same technique As is the case in obesity the boundaries of normal adipose tissue cellularity might be better defined when risk factors and diminished life-expectance can be included in the analysis for different parts of the spectrum In the present study the difficult question of normality has been circumvented by comparing obese subjects (V) with a randomly selected population (III Or Tables 2 and 4) To avoid a large frequency of drop-outs rapid and non-

traumatic methods had to be used. When the investigations in paper III and V were performed no whole body counter was available and the determination of exchangeable potassium required 36 hours of urine collection. Since glucose tolerance tests and adipose tissue biopsies also had to be performed it was considered unrealistic to determine body fat with the isotope technique. Instead anthropometric measurements were used to determine body fat by means of regression equations. High correlations were obtained between anthropometric measurements and body fat determined with isotope technique (III 16). The present reference material (III) demonstrates the associations between body fat, fat cell size and fat cell number. It is of particular interest that both fat cell number and fat cell weight correlate with body fat in the normal weight range while the correlation between fat cell weight and body fat can not be demonstrated in obese subjects (V 28-61).

No doubt random selection would have been preferable also in the analyses of adipose tissue cellularity in young men and women (IV). However the primary intention of paper IV was not to get control or normal values to compare with different pathological conditions but rather to search for differences in adipose tissue cellularity between normal young men and women. Such differences had been suggested in the middle-aged populations (Cf V) and it was considered easier to analyse this question in younger men and women. An adequate analysis of these problems however also required examination of the subcutaneous adipose tissue cellularity in different sites. The necessary measurements and other determinations were considered impossible to accomplish in randomly selected samples. Instead young healthy subjects with a weight index (present weight/ideal weight in relation to height) close to 1 was chosen in an attempt to measure the mean of the population. Unfortunately this selection probably resulted in samples which are not representative for the general population, the subjects being too tall and too slim. This is particularly obvious when these young highly selected men (IV) some of which were athletes are compared with medical students (III) which had not been preselected (Cf Table 2). However the selection probably allowed conclusions about the differences

in adipose tissue cellularity between the sexes (IV)

Obese subjects Obesity was arbitrarily defined as beginning at a body fat of 25 kg. The subjects were patients sent for general examinations at the First Medical Service, Sahlgren's Hospital.

There were no significant differences in adipose tissue cellularity between obese men and women (Table 2). Considering this and the limited number of subjects examined, obese men and women were pooled for the analysis of relationships between body fat, fat cell size and fat cell number. When the obese groups were compared to randomly selected subjects, women were chosen since most of the obese subjects were women. Also there were no statistically significant differences in fat cell size or number between the randomly selected men and women. This comparison made it possible to suggest a hypertrophic type of obesity associated with moderately increased body fat and a more severe hyperplastic obesity, the latter as a rule combined with fat cell hypertrophy.

In a simultaneous report Hirsch and Knittle did not present any obese subjects where hypertrophy contributed more than hyperplasia to body fat when comparing with a reference material (61). This can be explained by the fact that too few cases with moderate obesity were examined. While 21 of the 35 subjects in the present investigation (V) had a body fat below 50 kg, in Hirsch's material the corresponding figures were 2 of 90 subjects (61). If the same analysis as in Fig. 3 of paper V is performed on Hirsch's data, the y-axis will be located to the right of the intersection of the fat cell number line and the fat cell size line. Thus subjects with cellularity characteristics falling to the left of this y-axis were not studied by these authors.

Correlations In the present investigations positive correlations have been demonstrated between fat cell size and insulin levels in middle-aged men (III) and obese subjects (V). The lack of correlation in the comparatively few middle-aged women (III, 25) is not necessarily a female characteristic since positive correlations between fat cell diameter and fasting insulin in young women can be demonstrated.

by using primary data of study IV ($r=0.60$ $p<0.05$) Furthermore most of the obese subjects in paper V were women The association between fat cell size and insulin level has also been found in hyperlipidemic women (15) Though recent preliminary reports have confirmed the correlations between fat cell size and insulin levels (113-114) more studies are necessary in subjects of both sexes representing different physiological and pathological conditions So far the correlation has not been possible to demonstrate in maturity onset diabetes (18)

A further analysis of the relationship between obesity and plasma insulin levels in a larger group of subjects (108) preliminary indicate that weight change is a most important factor for the concentration of plasma insulin In obese subjects increasing in body fat over a period of about nine months the insulin levels were higher than in the obese subjects whose weights were unchanged during this time The lowest insulin values were found in obese subjects who had decreased in weight These findings might indicate that the correlations between insulin levels and fat cell size are associated with changes in the fat depot During weight gain increases in fat cell weight and insulin levels might be parallel phenomena whether mutually dependent or not The fact that physical training lowers insulin levels to normal in hyperinsulinemic obese subjects in spite of maintained or increasing body fat (15) suggests that increased fat cell size is not of primary importance for the development of hyperinsulinemia during weight gain in sedentary subjects Whatever the mechanism for the association between fat cell size and insulin level the stability of body weight apparently has also to be taken into account as an important variable

The Quantitative Importance of Fatty Acid Synthesis for Lipid Storage and Glucose Uptake in Human Adipose Tissue

The ratio of fatty acid synthesis de novo in man can be increased several times by high-carbohydrate feeding (VI-VII) The levels reached by stimulation are low in comparison with the fatty acid synthesis in adipose tissue of several other species (74-86) The

small amounts of fatty acids synthesized in whole cell systems (VI VII and review in I) could partly be explained by the dilution of labelled precursor by unlabelled compartmentalized precursor pools. While the maximum capacity for fatty acid synthesis of the enzymes involved is several times greater than the registered incorporation of glucose (VI VII) or glucose plus acetate (p 27) into fatty acids by whole cell preparations, it is still low from a quantitative point of view. During hypercaloric high-carbohydrate feeding the maximum capacity of the enzymes incorporating acetyl units into fatty acid on an average reached only 80 nmoles per g adipose tissue triglyceride and hour. Since there are no significant differences in this enzyme activity expressed per g triglyceride in different adipose tissue sites (p 25) or in different cellular types of adipose tissue (VI) it seems reasonable to make extrapolations from single adipose tissue preparations to the total adipose tissue mass. Then 80 nmoles/g adipose tissue TG hour corresponds to a synthesis of 0.06 g palmitic acid/kg body fat 24 hours. Assuming that 1 molecule of glucose produces 2 acetyl units this synthesis would require only 0.17 g glucose/kg body fat 24 hours. These are maximum values which indicates that fatty acid synthesis de novo in human adipose tissue most likely is low even in extreme conditions such as carbohydrate feeding of hyperinsulinemic subjects with a severe obesity. With a body fat of 50 kg only 3 g of palmitic acid per day would be synthesized corresponding to a glucose consumption of about 9 g. As discussed in paper VII these maximum figures are in good agreement with in vivo studies (11).

Apparently other fatty acids must play a major role in the process of lipid storage in human adipose tissue. This view is supported by the fact that lipoprotein lipase in human adipose tissue (for review see 89) has 100 to 1000 times larger capacity to make fatty acids available for storage than the enzymes involved in the synthesis of fatty acids de novo. Since the fatty acid synthesis also is low in human liver (100 101 107) it seems likely that exogenous fatty acids are of major importance for the storage of fatty acids in adipose tissue. This suggestion is supported by the fact that the pattern of fatty acids in adipose tissue tends to be similar to that in food (1 7 39 58).

GENERAL SUMMARY

The aim of the present investigation was to study the cellularity of human adipose tissue in relation to sex age and obesity Furthermore, the fatty acid synthesis de novo in adipose tissue was studied in relation to cellularity obesity and different diets These studies required new methods:

- 1 A microscopic method for the measurement of fat cells in frozen-cut adipose tissue was developed and compared to the electronic method of Hirsch and Gallian The correlation between the methods was high ($r=0.96$) Some improvements were made on the electronic method and a new calculation procedure was suggested The microscopic method was simpler faster and much cheaper
- 2 Using the method for fat cell size determination and ultra sound measurements a procedure was developed to estimate the local number of fat cells in perpendicular adipose tissue cylinders
- 3 A subcellular assay system was developed to determine the overall maximum capacity of the enzymes involved in fatty acid synthesis de novo in human adipose tissue by measuring the simultaneous incorporation of labelled acetyl units from acetate and citrate The activity of enzymes to incorporate acetyl units into fatty acids was almost exclusively recovered in the cytoplasmic fraction of the fat cell Several conditions were crucial for the assay system Linear incorporations with respect to time and enzyme concentration were attained The main products of the system were myristic and palmitic acids

With these methods the following results were obtained:

- 4 Fat cell size and number were determined in randomly selected middle aged men and women In both sexes fat cell size as well as number correlated with body fat Women had more body fat There were no differences in fat cell size or number between

young men and middle-aged men who had maintained a steady weight since they were 20 Middle-aged men who had increased in weight had more body fat than young men due to larger fat cells

- 5 Young women had more body fat than young men due to an increased total and local number of fat cells The distribution patterns of subcutaneous fat and local fat cell number in four examined regions were similar in men and women
- 6 In obese subjects fat cell number correlated with body fat while fat cell size did not In moderate obesity (25 - 40 kg of body fat) the greater amount of body fat compared to controls seemed to be caused by an increased cell size (hypertrophic obesity) With increasing obesity an increasing number of fat cells contributed proportionately more to body fat This hyperplastic obesity generally was combined with large fat cells
- 7 Significant positive correlations were found between the fasting insulin concentration and fat cell size in obese subjects and middle-aged men The sum of the insulin values during the oral glucose tolerance test and fat cell size also correlated
- 8 In vitro the capacity of the enzymes to incorporate citrate was at least as large as the capacity for acetate incorporation into fatty acids if optimal conditions for both precursors were used The supply of acetyl units and not the capacity of the enzymes synthesizing fatty acids is probably rate-limiting for fatty acid synthesis de novo in human adipose tissue
- 9 Expressed per g TG the overall activity of the enzymes involved in fatty acid synthesis was not different in different adipose tissue sites or in normal hypertrophic hyperplastic and combined hyperplastic hypertrophic adipose tissue Large fat cells were metabolically more active than small cells especially during carbohydrate feeding
- 10 Fatty acid synthesis and the enzymes necessary for this synthesis adapt to carbohydrate feeding Even the maximum levels reached are however of little quantitative importance for fatty acid storage or for glucose uptake in human adipose tissue

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APPENDIX

for calculations of fat cell size

A Microscopic Techniques

Method I.a Assuming that the measured cells are spheres the mean fat cell volume \bar{V} can be calculated from the measured diameters (μ) d_i as the average of all separate cell volumes using an IBM computer:

$$\bar{V} = \frac{\sum_{i=1}^{100} \left(\frac{\pi d_i^3}{6} \right)}{100} \mu^3$$

The mean fat cell weight \bar{W} is calculated in the same way using the density of human fat cell triglyceride (0.915 g/ml) (1) to convert the individual volumes to weights:

$$\bar{W} = \frac{\sum_{i=1}^{100} \left(\frac{\pi d_i^3}{6} \times 0.915 \right)}{100} \mu g$$

Method I.b With the aid of the average \bar{d} of 100 cell diameters and its standard deviation s \bar{V} and \bar{W} are calculated using an Olivetti computer (Programma 101) according to the formulas (2):

$$\bar{V} = \pi \left(\frac{s^2 \bar{d} + \bar{d}^3}{6} \right) \mu^3$$

$$\bar{W} = \pi \left(\frac{s^2 \bar{d} + \bar{d}^3}{6} \right) \frac{0.915}{10^6} \mu g$$

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If desirable the mean fat cell surface \bar{s} can be calculated with a similar formula (1):

$$\bar{s} = \pi (\bar{s}^2 + \bar{d}^2) \mu^2$$

B Osmium Fixation Techniques

The arbitrary volume (V) of particles measured in an electronic particle counter (Coulter Counter Model B or Celloscope 302) is:

$$V \approx \frac{D}{A} \frac{1}{I} \quad 1)$$

where D = setting of discrimination

A = amplification

I = aperture current

The true volume (v) and diameter (d) of particles can be written:

$$v \approx V K_v \quad 2)$$

and

$$d \approx \sqrt[3]{V} K_d \quad 3)$$

where K_v and K_d are constants

The counter is calibrated by using particles (corn pollen) with a known mean diameter (\bar{d}). The settings of D , A and I at which 50% of these particles are counted is considered to correspond to the known mean diameter value \bar{d} . Using the values of D , A and I and equations 1) - 3) it is possible to calculate K_v and K_d :

$$K_v = \frac{v}{V} \frac{\bar{d}^3}{\bar{d}^3} \quad \frac{A}{D} \frac{I}{I} \quad 4)$$

$$K_d = \bar{d} \sqrt[3]{\frac{A \cdot I}{\pi}}$$

5)

When values of K_v and K_d are known formula 2) and 3) can be used for calculation of the true volume (v) and its diameter (d) respectively from any combination of D , A and I . The constants must be determined for each individual counter or capillary tube.

For each sample the number of cells per ml suspension are determined in quadruplicate at different settings corresponding to a geometrical series of volumes. By increasing the sensitivity of the counter the detectable volume decreases with a factor of $\sqrt{2}$ in each step of this series.

Coincidental passage through the capillary tube is corrected for according to Wales and Wilson (1). One and occasionally two dilutions of the suspension are performed while counting. Dilution factors are obtained by determining the number of counts at the same settings before and after dilution. From these determinations a cumulative curve of fat cell volumes or diameters is constructed. Calculation of the mean fat cell size is then performed by (one of) the following two procedures:

Method IIa: The mean fat cell weight (\bar{w}) expressed as the weight of the mean lipid content (μg) is calculated according to the formula:

$$w = \frac{\left(\begin{array}{l} \text{wet weight of sample} \\ \text{for osmium fixation } (\mu g) \end{array} \right) \left(\begin{array}{l} \text{ratio of lipid} \\ \text{to wet weight} \end{array} \right)}{\left(\begin{array}{l} \text{cells per ml} \\ \text{(ml of suspension)} \end{array} \right)} \quad (6)$$

This calculation procedure is identical with that of method III of Hirsch and Callian (2).

Method IIb: The number of counts per ml between all adjacent settings (ΔN) is calculated as well as the corresponding mean arbitrary volumes (\bar{V}). Since the V values constitute a geometrical series which decreases with a factor of $\sqrt{2}$ in each step, the V values form a similar series. The product $\Delta N \cdot \bar{V}$ represents the total arbitrary volume of all cells in 1 ml suspension between two adjacent settings. Thus $\sum \Delta N \cdot \bar{V}$ is the total arbitrary volume of all cells in 1 ml suspension. The mean arbitrary fat cell volume (\bar{V}) is obtained by

1. Wales M. & Wilson J W : Rev Scientific Instr 32:1132 1961
2. Hirsch J & Callian E : J Lipid Res 9:110 1968

dividing the total arbitrary volume with the number of cells constituting it that is the number of cells per ml suspension (N):

$$\bar{V} = \frac{\sum \Delta N}{N} \quad (7)$$

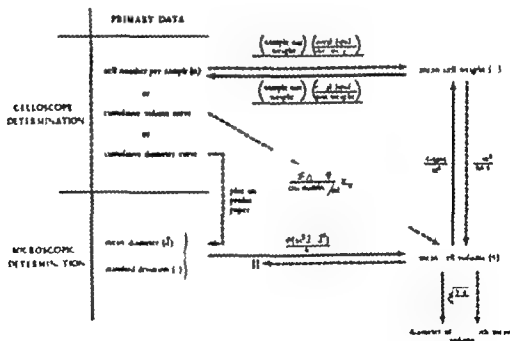
The true mean volume (\bar{V}) of the fat cells is obtained from the calibration of the counter (Cf formula 2));

$$\bar{V} = \bar{V} \cdot K_v \cdot \mu^3 \quad (8)$$

The mean fat cell weight (\bar{w}) is calculated from the volume using the density of human adipose tissue triglycerides:

$$\bar{w} = \frac{\bar{V} \cdot 0.915}{10^6} \mu g \quad (9)$$

Interrelationships between different fat cell characteristics are shown below



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Respiratory and Circulatory Investigations in Obstructive and Restrictive Lung Disease

**By
Senefro K Gabriel**

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INTRODUCTION

The trend towards extensive industrialization and the creation of modern industrial societies have contributed to the increase of chronic non-specific lung disease and occupational lung diseases. Chronic obstructive lung disease, or chronic bronchitis/emphysema, is becoming the most prevalent chronic lung disease in the United States and the mortality attributed to this complex has increased sixfold in a period of 10 years (40). In Great Britain 10 % of death among men between 40—65 years are caused by chronic bronchitis (40). In most countries the increased incidence of this complex and of the occupational lung diseases is becoming a significant factor also in physical disability. In consequence the diagnosis of these diseases is becoming important. However the problems and difficulties of radiological diagnosis and the discrepancy between radiological and pathological changes demand an elaborate evaluation of pulmonary function to assist in deciding on medico-legal problems.

A large number of analytical methods have been developed, aimed in the first place at determining the pathophysiological changes of respiratory diseases. In

the course of time the number and complexity of these methods have increased considerably. The use of pulmonary function tests in the clinical investigation of patients with lung disease has proved to be of a high diagnostic and therapeutic value, and there has therefore been an increased demand by clinicians for these tests. In order to be able to meet these requests and apply the tests to as large a number of patients as possible, it seemed to be of importance to restrict the number of methods in use.

The aim of the present study was to evaluate some of the current methods in order to know which of them have the best practical discriminatory value and to allow an accurate quantitation of physiological derangements. It was also desired to get further information about some of the pathophysiological changes, which will be also reported.

The material for the present study is composed of patients with chronic obstructive and restrictive lung disease. Different methods were applied and a comparison was made for each method. On the basis of this comparison a suitable investigation procedure has been proposed.

CHAPTER I

MATERIAL AND METHODS

Material

The material comprised patients with chronic lung disease referred to this laboratory for routine cardiopulmonary functions tests. The aim in the selection was to collect two groups of patients functionally classified into A) patients with chronic obstructive lung disease, B) patients with chronic restrictive lung disease. Patients with primary heart disease or pneumonectomy were excluded. The following criteria were applied

1) *Clinical criteria.* a) *Chronic bronchitis* History of chronic productive cough for a minimum of three months per year and for at least two years (American Thoracic Society) (1)

b) *Bronchial asthma* was diagnosed whenever the patient had a history of acute attacks of dyspnea and wheeze.

c) *Emphysema.* The diagnosis was based on the following radiological criteria 1. hyperaeration of the lungs, 2. flat tented diaphragm, 3. reduced vascular shadow. In some patients the radiological diagnosis was combined with clinical and physiological findings.

d) *Pulmonary fibrosis and pleural thickening* The diagnosis was based on clinical, radiological and laboratory investigations.

2) *Functional criteria.* For functional classification the material was divided into two groups.

A) *Obstructive lung disease* FEV % $< x - 1$ SD where x is the predicted normal value according to Berglund *et al.* (3) In three patients (Nos. 5, 9 and 16) FEV % was between $x - 1.5$ SD and $x - 2$ SD in all other patients FEV % was $< x - 2$ SD. No regard was paid to the vital capacity

B) *Restrictive lung disease* Vital capacity (VC) < 80 % of the predicted normal value and normal FEV % Cases 8, 12, 13 16 and 17 had a decreased FEV % as well. These patients were included in the group, as they had predominantly restrictive impairment as judged from the spirometric data.

Group A with obstructive lung disease included 24 patients, 16 men and 8 women, with one or more of the following diseases: chronic bronchitis, emphysema or asthma. Seven patients (Nos. 1 4 9 10 17 18 and 19) had old healed pulmonary tuberculosis as well. However the radiological evidence of the disease was minimal and the patients' complaints were not related to it. In the 5 asthmatic patients the disease had extended over many years, and was associated with chronic bronchitis and/or emphysema. Except for case 24 they had had at one time or another a positive skin allergen test.

Group B with restrictive lung disease included 17 patients, 12 men and 5

women. Eight patients had pulmonary fibrosis of various etiology and nine had pleural disease. In eight patients the pleural disease was secondary to old pulmonary tuberculosis, of whom 4 had pronounced pleural thickening, 3–7 cm and 4 minor pleural thickening of these 8 patients 6 had been treated with artificial pneumothorax and two with thoracoplasty. In one patient (No. 11) the pleural thickening was secondary to influenza empyema. He also had a lyphoc deformity of the thoracic spine. For descriptive purposes the term pleural restriction will be used in referring to these patients. The main clinical diagnoses are given in Tables I–II Chapter II

Methods

Total amount of hemoglobin (THb g) was determined by the alveolar carbon monoxide method according to and described by Sjstrand (32–33). *Hemoglobin concentration (Hb g/100 ml)* was measured spectrophotometrically (16).

Blood volume (BV l) was calculated from THb and hemoglobin concentration of fingertip blood and corrected for the systemic difference between the total body hematocrit and peripheral vessel hematocrit using the factor 0.91. For review see (6).

Heart volume (HV ml) was determined in the prone position according to Larsson and Kjellberg (22) with the modification that the X-ray tube was inclined 30° caudally (21).

Static spirometry. Total lung capacity (TLC) and its subcomponents: total capacity (VC), residual volume (RV), functional residual capacity (FRC) and

ratio of residual volume to total lung capacity (RV/TLC %) and the ratio of functional residual capacity to total lung capacity (FRC/TLC %) — were determined in sitting position using a closed circuit helium dilution spirometer (14) (Kjfa, Stockholm). Normal values were predicted according to Grimby and Soderholm (12). All values were converted to BTPS using the factor 1.1.

Dynamic spirometry (1 BTPS)

Forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), FEV₁ %

$\left(\frac{FEV_1}{VC} \times 100\right)$, forced inspiratory volume in one second (FIV₁), maximal voluntary ventilation at a respiratory rate of 40 (MVV₄₀) and maximal voluntary ventilation at a free respiratory rate (MVV_{free}) were determined with a Bernstein spirometer (Kjfa, Stockholm) (4). Normal values were predicted according to Berglund *et al.* (3).

Orthostatic test. Heart rate was determined in the supine position after 10 minutes rest and after 11 minutes standing erect from an ECG record.

Exercise tolerance test. The exercise tolerance test was performed according to Sjstrand (31) and Wahlund (36) on an electrically braked cycle ergometer (15) in sitting and supine position on two different occasions, starting at a rate with 200 kpm/min and increasing the work load stepwise at the end of 6 minutes. Heart rate was determined every two minutes at each work load. The respiratory rate during one minute was determined by auscultation during the third minute of each work period. All patients were urged to continue the

work test until disabling symptoms or signs appeared. The rate of work at a heart rate of 130 beats/min (W_{130}) was determined by inter or extrapolation using an approximately linear relationship between the heart rate and the work load. No regard was paid to the presence of steady state.

The rate of work at the exercise breaking point (W_B) This measure is the same parameter as W_{max} suggested by Strandell (35). In lung disease, however maximal performance may be affected by external factors, which is why this term was preferred. It was calculated as the highest work load the patient could sustain for 6 minutes with the addition of the load for the next non-completed period after recalculation to refer to 6 minutes. Example: A patient completes the first work period of 6 minutes at a rate of work of 300 kpm/min, while in the next period he interrupts the test after two minutes at a work load of 600 kpm/min. his W_B will be equal to 300 (work load of the first period $+ 2/6 \times 300$ (work load of the second period) = 400 kpm/min. The breaking point heart rate (HR_B) was taken as the highest heart rate recorded during exercise. All heart rates were determined from ECG records.

Diffusing capacity of the lung for carbon monoxide (D_{LCO} , ml/min/mm Hg) was determined by a steady-state technique according to Filley *et al.* (10) with correction for CO capillary back pressure according to Linderholm (24). D_{LCO} was calculated according to the equation $\frac{V_{CO}}{P_{ACO} - P_{ECO}}$ where V_{CO} is the

carbon monoxide uptake, P_{ACO} the alveolar carbon monoxide tension, P_{ECO} the carbon monoxide tension in the pulmonary capillaries. The measurements were performed during steady-state exercise on a cycle ergometer in the sitting position. The work load was selected individually from previous exercise tolerance tests to increase the heart rate to ≥ 120 beats/min. Fractions of gases F_O and F_{CO_2} were analysed by the Haldane technique. Gas volumes were measured with a spirometer. Carbon monoxide in gas phase was analysed with a Hopcalite CO meter (Stålex) as described by Linderholm and Sjöstrand (23). The carbon monoxide content in blood was determined by the method described by Linderholm, Sjöstrand and Söderström (25). The carbon monoxide capacity of blood was taken to be equal to 1.34 ml/g Hb.

The reproducibility of measurements of D_{LCO} during exercise in this laboratory is between 7.2–6.4% (18, 19).

Alveolar ventilation (V_A , l/min) and alveolar oxygen tension were calculated from the alveolar gas equation. Physiological dead space (V_D , ml) was calculated using the Bohr's equation and assuming the alveolar carbon dioxide tension to be equal to the arterial carbon dioxide tension (9, 28). The respiratory valve had a dead space of 40 ml at rest and 50 ml during exercise.

Oxygen uptake (V_{O_2} , ml/min) carbon dioxide elimination (V_{CO_2} , ml/min) and D_{LCO} were expressed at STPD.

Total ventilation (\dot{V}_E , l/min) V_A and V_D were expressed at BTPS.

The veno-arterial shunt (Q_A) was measured according to Bergeren (2) during 100 % oxygen breathing in a prone position until the nitrogen concentration in the expired air reached a level of 2 %. The calculation was made according to the equation

$$\frac{Q_A}{Q} = \frac{C_{\text{end}} - C_{\text{ven}}}{C_{\text{end}} - C_{\text{ex}}}$$

Where C_{end} , C_{ven} and C_{ex} were end-capillary, arterial and mixed venous blood oxygen contents and Q was the systemic blood flow.

The arteriovenous oxygen difference was taken to be 4 volumes %. In our laboratory shunt values up to 7 % of the cardiac output are considered normal with this technique.

Arterial oxygen tension (P_{aO_2} , mm Hg) was determined with a Clark electrode.

Arterial carbon dioxide tension (P_{aCO_2} , mm Hg) was measured with a glass electrode according to Severinghaus. Instrumental in Laboratory Inc. blood gas analyzer model 113.

pH was determined by the Astrup titrimetric method.

Oxygen saturation was determined spectrophotometrically by the method of Dumas et al. (1961).

Cardiac output was determined by the Fick method using the oxygen consumption and arterial-venous oxygen difference.

Intravascular pressures were measured with Elema differential transformer transducers (EMT 490 A). About half of the material was recorded on an Elema "Klinik" recorder and the other half with Medec strain gauge transducers (30-145) and an UV recorder (Oscillofil, Siemens). Mean pressures were obtained by electrical integration (time constant 1.0 second). The pressure reference level was the midthoracic point of the anteroposterior diameter at the sternal insertion of the fourth rib. Cardiac output (Q) was measured according to the direct Fick method. The pulmonary vascular resistance index (PVR) was calculated as the difference between the pulmonary artery mean pressure and the pulmonary wedge pressure divided by the cardiac index (C.I.) (cardiac output divided by body surface area). The systemic vascular resistance index (SVR) was measured as the brachial artery mean pressure divided by the cardiac index. Expired air was collected in Douglas bags, usually for 8 minutes at rest and 3 minutes during exercise. Gas volumes were measured with a spirometer. Expired gas was analyzed for oxygen and carbon dioxide by the Haldane technique.

Errors for a single determination estimated from duplicate determinations and the reproducibility of the method are the same as reported in other studies from this laboratory (7, 20).

Statistical Methods and Computer Programmes

Statistical methods and computer programmes were performed using the SPSS (Statistical Package for the Social Sciences) (1979, 34) Data

ferences between regression lines were tested according to Hald (1960) (13). The following probability levels of significance were used

$p \leq 0.001$ highly significant

(***)

$0.001 < p \leq 0.01$ significant (**)

$0.01 < p \leq 0.05$ prob. significant (*)

II Computer programmes

Diffusing capacity and ventilation were calculated on an IBM 7044 computer. Correlation matrix and multiple regression analysis were performed with the IBM 1130 computer at the computer centre at Karolinska Institute. Other computations were performed at this department with Olivetti, Electronic desktop computer programme 101

Definitions, Symbols and

Abbreviations

Definitions, symbols and abbreviations used were mainly as recommended by Gandevia and Hugh Jones (11) and Pappenheimer *et al.* (27)

Procedure

All patients were hospitalized during the period of examination, during which routine clinical and laboratory investigations were made. Functional studies started with a determination of total amount of hemoglobin, blood volume, heart volume, two exercise tolerance tests, one in

sitting and the other in supine position on two separate occasions, ECG phonocardiogram and spirometry. Ventilation, diffusing capacity and shunt studies were performed on a separate day as was also the heart catheterization. The procedures used during the measurements of diffusing capacity and in heart catheterization are reported in chapters IV and V respectively

Comments

The methods adopted in the present investigations are well established, though their application in the present studies might be subject to criticism. For instance the calculation of $P_{A_{O_2}}$ from the alveolar air equation leads to an error in the presence of uneven ventilation/perfusion, as an arterial alveolar CO_2 tension difference may exist. This results in underestimation of $P_{A_{O_2}}$ and other related variables. Also Mellergaard (26) has shown that the method of Berggren leads to over-estimation of the veno-arterial shunt in patients with maldistribution of ventilation. However it is assumed that the error which might be introduced will be reflected proportionally in both groups and thus for comparative purposes, it will not significantly influence the results expected.

References are given in the next chapter

CHAPTER II

LUNG VOLUMES, VENTILATORY CAPACITY and CIRCULATORY DIMENSIONS

Results

Clinical and anthropometric data are given in Tables I—II Spirometric data are
 Table I Clinical and anthropometric data in group A. 4 patients, 16 men and 8 women with

Subject no.		Sex	Age yrs	Height m	Weight kg	B.S.A. m ²
1	L.A.	M	5	168	51.5	1.59
2	K.T.	M	11	174	60.5	1.75
3	H.T.	M	63	163	66.5	1.7
4	E.E.	M	64	174	69.5	1.85
5	W.A.	M	40	181	69.0	1.9
6	R.K.	M	40	181	74.0	1.95
7	S.J.	M	53	176	77.0	1.91
8	W.M.	M	65	177	85.5	2.0
9	C.T.	M	67	178	54.0	1.70
10	H.M.	M	61	17	53.5	1.68
11	F.E.	M	69	177	50	1.89
12	A.G.	M	55	165	55.0	1.60
13	J.L.	M		185	56.0	1.99
14	A.T.	M		171	56.0	1.70
15	A.W.	M	71	17	69.0	1.8
16	P.T.	M	6	161	49.5	1.51
17	S.T.	F	15	160	51.0	1.5
18	F.K.	F	7	161	50	1.60
19	E.A.	F		150	55.0	1.48
20	B.W.	F	1	161	60	1.55
21	A.T.	F	2	163	57.0	1.61
22	E.E.	F	48	1	41.0	1.40
23	A.S.	F	1	16	41.0	1.40
24	L.H.	F	20	174	71.0	1.77
Men						
			5	173	65.9	1.8
SD			11.5	0.03	11.6	0.15
Range			5	163-185	4	50-85
			1	1	16	1.6
Women						
			15	161	51	1.51
SD				4.0	6.1	0.1
Range			1	150-181	41	1.40-1.77
				1	6	1.6

given in Tables IV—VI
chronic obstructive lung disease.

Total hemoglobin g	Blood volume l	Heart volume supine ml	Clinical diagnosis
595	5.0	610	asthma + chronic bronchitis
700	5.8	985	emphysema
601	4.8	750	emphysema
472	4.9	640	chronic bronchitis + emphysema
568	4.8	760	emphysema
562	4.4	890	chronic bronchitis
720	5.6	860	emphysema
736	5.3	1020	asthma + chronic bronchitis + emphysema
477	4.5	650	emphysema
458	5.8	505	emphysema
523	5.8	615	emphysema
465	3.3	1145	chronic bronchitis + emphysema + secondary polycythemia
606	4.0	810	emphysema + cardio-sclerosis + secondary polycythemia
423	3.4	596	chronic bronchitis + emphysema
700	5.5	805	chronic bronchitis
444	4.0	435	emphysema
488	3.9	575	asthma + emphysema
498	4.2	550	chronic bronchitis + emphysema
415	3.5	—	asthma + emphysema
408	3.3	695	chronic bronchitis + emphysema
585	3.2	680	asthma + chronic bronchitis
347	3.2	525	chronic bronchitis
581	3.9	—	emphysema + fibrosis
566	5.6	730	asthma + chronic bronchitis
564	4.6	757	
107	0.78	193	
425—736	3.3—5.8	435—1145	
16	16	16	
436	3.9	626	
74	0.80	86	
347—566	3.2—5.6	525—730	
8	8	6	

Table II Clinical and anthropometric data in group B. 17 patients, 11 men and 5 women, with

Subject no.		Sex	Age years	Height cm	Weight kg	B.S.A. m ²
1	H.B.	M	60	173	57.0	1.70
2	E.E.	M	4	187	68.0	1.90
3	R.U.	M	49	170	64.0	1.73
4	V.S.	M	61	167	62.0	1.70
5	J.S.	M	48	171	74.0	1.87
6	D.F.	M	37	181	53.5	1.75
7	G.Z.	M	0	176	64.5	1.80
8	E.O.	M	49	182	67.5	1.90
9	E.P.	M	50	177	81.5	2.00
10	C.M.	M	67	183	87.0	1.9
11	L.H.	M	77	162	62.0	1.67
12	A.R.	M	5	173	79.0	1.93
13	C.J.	F	31	168	79.0	1.89
14	S.H.	F	40	151	65.5	1.62
15	H.B.	F	49	157	54.5	1.51
16	A.D.	F	30	166	51.5	1.60
17	L.P.	F	65	161	60.5	1.68
Men						
—			7	171.8	68.9	1.81
SD			10.36	6.63	10.26	0.14
Range			4—77	16—183	53.5—87.0	1.62—2.10
			1	1	1	1
Women						
—			48	161.0	63.8	1.67
SD			11.6	5.89	10.12	0.15
Range			31—65	14—168	51.5—79.0	1.51—1.89
			5	5	5	5

Table III Anthropometric data differences between group means.

Value	Sex	Group A	Group B	P
Age (years)				
M		60	7	—
F		53	48	—
Height (cm)				
M		173.5	171.8	—
F		160.1	161	—
Weight (kg)				
M		79.9	63.9	—
F		55	63.8	—
B.S.A. (m ²)				
		1	1.71	—
		1	1	—

chronic restrictive lung disease.

Total hemoglobin g	Blood volume l	Heart volume supine, ml	Clinical diagnosis
487	4.2	504	left pl. thickening + lobectomy and thoracoplasty
633	4.4	502	left pl. thickening + right thoracoplasty
653	5.7	646	pulmonary fibrosis (ch. interstitial fibrosis)
600	5.3	990	pulmonary fibrosis (ch. interstitial pneumonia)
628	4.9	850	pulmonary fibrosis (silicosis)
563	6.3	610	right pleural thickening
477	4.7	805	pulmonary fibrosis (silicotuberculosis)
610	6.6	600	bilateral pleural thickening
780	6.4	885	pulmonary fibrosis (asbestosis)
734	5.8	970	left pleural thickening
608	4.9	—	left pleural thickening + left contracted lung
829	6.6	790	left pleural thickening + left contracted lung
594	4.6	770	left pleural thickening + left contracted lung
565	4.5	680	pulmonary fibrosis (pulmonary vascular hyperplasia)
562	3.2	630	pulmonary fibrosis + chronic bronchitis
418	3.8	430	pulmonary fibrosis (post irradiation)
534	4.1	620	right pleural thickening
635	5.5	741	
106	0.75	177	
477—829	4.2—6.6	502—990	
12	12	11	
493	4.0	626	
100	0.57	125	
562—594	3.2—4.6	430—770	
5	5	5	

Variable	Sex	Group A	Group B	P
Total hemoglobin (g)	M	564	635	—
	F	436	493	—
Blood volume (l)	M	4.6	5.5	<0.01
	F	3.9	4.0	—
BV/kg (ml)	M	72.5	81.0	—
	F	72.3	63.6	—
Heart volume, supine (ml)	M	757	741	—
	F	626	626	—

Table 11. Spirometric data in group A. For symbols, see methods.

Subject no.	Sex	TLC	VC		RV	FRC	
			l	pred.			
1	U A.	M	3.25	3.38	70	1.67	3.23
2	K.E.	M	11.10	4.29	89	6.81	8.90
3	H.F.	M	6.77	3.6	85	3.15	3.98
4	L.F.	M	6.75	2.8	60	3.41	4.17
5	W.A.	M	9.43	4.46	41	6.97	8.33
6	R.K.	M	6.48	3.97	71	2.57	3.85
7	S.J.	M	6.80	3.91	6	81	3.91
8	W.M.	M	6.7	86	9	4.06	4.6
9	G.F.	M	3.75	.67	53	3.18	4.39
10	H.M.	M	8.05	.67	5	3.41	6.70
11	E.F.	M	7.47	3.30	1	4.16	5.03
12	A.C.	M	3.27	.08	47	3.19	3.89
13	J.I.	M	9.33	3.41	68	3.97	7.1
14	A.F.	M	6.01	1.91	41	4.07	4.87
15	V.W.	M	7.11	4.21	95	3.20	4.6
16	I.F.	M	.99	.67	75	3.33	4.47
17	S.E.	F	4.72	1.90	57	82	3.49
18	F.K.	F	5.18	.08	63	3.10	3.77
19	F.A.	F	3.67	.41	95	1.6	.11
20	B.W.	F	4.68	1.91	5	7	3.70
21	A.F.	F	4.1	1.60	49	2.91	3.77
22	F.P.	F	4.10	.33	7	1.77	2.00
23	A.S.	F	4.71	1	4	3.09	3.51
24	L.H.	F	5.11	.39	6	55	5.1
Men 16	\bar{x}	7.14	3.15	67	4.00	5.11	
	SD	1.63	0.74	15.50	1.5	1.69	
	Range	5.1-11.10	1.91	1-95	1.6-6.81	3.3-8.90	
Women 8	\bar{x}	4.58	.01	45	3.1	3.1	
	SD	0.70	0.33	17.05	0.67	0.51	
	Range	3.6-5.18	1.5	5-95	1-7	1.1-3.77	

Lung volumes and ventilatory capacity

Group A Stat. sig. volumes /
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161 cc and in women 181 cc of predicted values ($p < 0.001$). FRC was increased in both sexes and in men averaged 192 cc ($p < 0.05$) and in women 131 cc ($p < 0.001$) of predicted values. The RV/TLC cc ratio averaged in men 55 cc and the FRC/TLC 71 cc these values being signi-

$\frac{RV}{TLC} \%$	$\frac{FRC}{TLC} \%$	FVC	FEV ₁	%	FEV % % pred.	$\frac{FEV_2}{FEV_1}$	MVV ₄₀	MVV _{free}
32	6	3.11	1.98	59	74	0.76	49	57
61	80	2.61	1.32	34	49	0.61	32	39
47	59	3.85	1.95	48	68	0.56	68	97
53	67	2.12	0.80	36	54	0.47	20	17
74	88	3.91	2.34	60	81	0.86	58	71
40	59	4.45	3.00	66	86	0.71	92	11
42	58	3.52	1.50	38	55	0.44	68	56
59	69	2.00	0.85	33	49	0.47	29	27
55	76	2.90	1.45	30	75	0.67	39	58
67	83	1.75	0.70	29	42	0.39	19	19
56	67	2.65	1.10	32	48	0.32	41	43
61	74	1.45	0.70	35	49	0.32	25	26
63	76	1.85	0.80	25	39	0.27	29	30
68	80	1.50	0.95	44	61	0.68	15	16
43	64	4.10	1.45	35	34	0.45	62	81
56	75	2.80	1.60	54	81	0.54	43	46
60	74	2.09	1.21	58	73	0.59	51	34
60	73	1.10	0.58	30	39	0.45	19	24
31	57	2.20	1.16	53	71	0.62	34	39
59	79	1.75	0.63	33	42	0.65	16	16
65	72	1.40	0.70	30	65	1.06	14	19
71	71	1.90	1.45	64	80	0.83	46	69
67	77	1.08	0.70	46	59	0.61	26	26
50	61	1.75	0.95	37	47	0.42	23	16
55	71	2.79	1.41	42	60	0.53	43	50
11 43	9 28	0.97	0.66	12.35	15.14	0.17	21 63	28.87
32-68	58-83	1.45-3.91	0.70-3.00	21-71	25-66	0.27-0.86	15-92	16-112
55	71	1.66	0.93	46	60	0.66	26	30
11 46	7 63	0.43	0.32	12.18	13.34	0.21	10 63	17.65
34-67	57-79	1.08-2.20	0.58-1.21	30-64	39-80	0.42-1.06	14-46	16-64

significantly high in both sexes ($p < 0.001$)

Dynamic lung volumes (1 BTPS)

Mean FVC was slightly lower than mean VC in both sexes, the difference being non-significant in men ($p > 0.05$) while in women it was probably significant ($p < 0.05$). FEV, FEV₁, MVV₄₀ and MVV_{free} were all decreased in both

sexes ($p < 0.001$). The ratio FEV₁/FIV₁ was 0.53 in men and 0.66 in women both values were low compared to 0.88 found in normal subjects by Simonson (30).

Group B Static lung volumes (1 BTPS) VC and TLC were significantly decreased in both men and women

Table 1 Spirometric data in group B. For symbols, see methods.

Subject no.	Sex	TLC	VC		RV	FRC	
			l	% pred.			
1	H.B	M	3.20	93	61	2.27	3.47
	E.E.	M	3.39	29	40	1.37	2.17
3	R.U	M	3.41	3.69	6	1.73	3.01
4	V.S	M	3.60	41	31	1.16	.38
5	J.S	M	3.53	2.7	46	1.28	.09
6	B.F	M	3.46	89	33	.7	3.93
7	C.Z	M	4.40	3.33	71	1	3.01
8	F.O	M	3.91	2.29	42	1.65	.79
9	E.P	M	4.31	1.0	33	.61	3.18
10	C.V.L	M	3.57	3.4	67	.15	3.4
11	I.H	M	4.67	1.80	47	2.87	3.51
12	V.R.	M	3.01	3.49	7	1.53	.59
13	C.J	F	3.79	1.90	47	1.39	1.9
14	S.H	F	.47	1.83	31	0.11	1.07
15	H.B	F	.71	0.93	99	1.81	.09
16	A.D	F	3.38	1.68	47	1.49	.39
17	I.P	F	3.3	1.81	60	1.44	.29
Men		—	4.61	.71	—	1.49	3.0
n = 17	SD	0.8	0.19	14.16	0.48	0.61	
	Range	3.3—5.57	1.0—3.6	33—1	1.16—87	.09—3.93	
Women		—	3.01	1.63	48	1.13	1.93
	SD	0.43	0.40	11.93	0.40	0.53	
	Range	2.47—3.58	0.93—1.49	31—60	0.11—1.1	1.07—3.9	

($p < 0.001$) RV averaged 80% of the predicted value ($p < 0.05$) in men and 101% in women ($p > 0.05$) FRC was on average 3% of the predicted value in men ($p < 0.001$) and 88% in women ($p > 0.05$). As the reduction in TLC was greater than the reduction in RV and FRC, the ratios of the two latter to the former were increased.

Dynamic lung volumes (1 BTPS)
 FEV₁ averaged 33% of the predicted value in men and 81% of the predicted value in women. In both sexes the difference between observed and predicted

values was non-significant ($p > 0.05$) FEV₁, MVV_{max} and MVV_{100%} were significantly decreased in both sexes. The ratio FEV₁/FEV₁ averaged in men and women 0.80 compared to 0.88 reported by Simonsson in normal subject (30).

From Tables IV and V it can be seen that in groups A and B some patients had higher MVV_{max} than MVV_{100%}. The significantly small dynamic lung volumes in group A and VC and TLC in group B were partly influenced by the criteria of selection.

$\frac{RV}{TLC} \%$	$\frac{FRC}{TLC} \%$	FVC	FEV	$\%$	FEV % % pred.	$\frac{FEV_1}{FEV_1}$	ΔRV_{40}	ΔRV_{100}
44	67	2.58	1.81	67	96	0.63	63	78
38	60	2.31	1.60	69	91	—	49	58
52	67	3.85	2.97	76	103	0.80	100	143
52	66	2.50	1.65	66	96	0.87	48	92
56	59	2.23	1.57	70	96	0.70	56	81
47	72	2.70	1.87	68	97	0.72	71	95
51	62	3.40	2.00	59	88	1.08	41	45
42	71	2.25	1.15	49	67	0.59	57	51
61	74	2.50	1.45	85	116	1.52	37	55
89	62	3.40	.50	69	103	0.76	84	108
62	76	1.75	0.95	54	86	0.59	26	28
51	61	3.14	1.98	57	79	0.68	52	57
42	60	1.54	1.02	55	66	0.56	35	46
26	41	1.76	1.65	86	106	0.98	56	45
66	76	0.95	0.70	64	81	0.88	16	36
55	67	2.10	1.55	74	94	0.94	38	44
44	70	1.40	0.95	54	72	0.63	31	38
41	66	2.72	1.79	66	95	0.80	55	75
10.81	5.78	0.61	0.54	9.83	12.51	0.22	21.24	35.41
31—62	49—76	1.75—3.85	0.95—2.97	49—85	67—116	0.59—1.52	26—100	28—143
46	63	1.55	1.17	67	84	0.80	55	42
14.74	13.48	0.49	0.41	15.52	16.28	0.19	14.58	4.49
26—66	41—76	0.95—2.10	0.70—1.65	54—86	66—106	0.56—0.98	16—56	36—16

Table 17 Comparison between groups A and B spirometric measurements as percentage of predicted normal values.

		TLC % pred.	VC % pred.	RV % pred.	FRC % pred.	FEV % pred.	FEV % % pred.	ΔRV % pred.
Men	A = 16	99	66	161	122	42	60	38
	B = 12	63	55	80	73	52	58	47
Women	A = 8	100	65	184	134	57	60	30
	B = 5	64	48	104	88	43	64	38
p	Men	<0.001	>0.05	<0.001	<0.001	>0.05	<0.001	>0.05
A—B	Women	<0.001	>0.05	<0.01	<0.01	>0.05	<0.001	>0.05

Total hemoglobin blood volume hemoglobin concentration and heart volume

Total amount of hemoglobin in group A averaged in men 8.92 g/kg body weight, SD ± 1.46 and in women 8.11 g/kg body weight, SD ± 0.88 . In group B the average for men was 9.28 g/kg body weight, SD ± 0.96 and for women 7.72 g/kg body weight, SD ± 0.76 . The mean values for men in both groups were significantly lower than normal values, for A $p < 0.001$ and for B $p < 0.01$. In our laboratory (8) normal values are 10.23 ± 1.18 g/kg for men and 8.07 ± 0.85 g/kg for women.

Blood volume in group A averaged in men 77.3 ml/kg body weight, SD ± 17.8 and in women 72.3 ml/kg body weight, SD ± 11.9 . The values for men were slightly lower than normal values, $p < 0.05$. In group B the average for men was 81.0 ml/kg body weight, SD ± 13.0 and for women 63.5 ml/kg body weight, SD ± 5.4 . The males values did not significantly deviate from normal values while the female values were significantly lower, $p < 0.01$. Normal values are for men 82.4 ± 8.9 ml/kg and for women 73.9 ± 7.5 ml/kg (8).

Hemoglobin concentration in group A averaged in men 13.5 g/100 ml (range 11.5–16.0) and in women 12.5 g/100 ml (range 11.2–13.8). In group B it was 12.8 g/100 ml in men (range 11.5–16.0) and 12.5 g/100 ml (range 10.1–14.1) in women.

Heart volume in group A averaged in men 676 ml (range 430–770) and in women 626 ml (range 430–770). In group B the average for men was 676 ml (range 430–770) and for women 626 ml (range 430–770). The males values did not significantly deviate from normal values while the female values were significantly lower, $p < 0.01$. Normal values are for men 676 ± 178 ml and for women 626 ± 119 ml (8).

676 ml (range 430–770) for women. In group A the individual values varied by 2 SD in relation to THb, except in six patients (Nos. 2, 6, 8, 12, 20 and 21) (Fig. 1). In group B the heart volume was normal in relation to THb, except in two patients whose volume was above 2 SD (Nos. 4 and 7) (Fig. 2). Both patients had signs of cardiovascular disease. However the values in both groups were within ± 2 SD (except in one patient A 12) when compared to the regression line found in elderly normal subjects aged 56–83 (35). In both groups the mean heart volume was between the regression lines of the young and elderly subjects which is normal for the age of the groups. However in the restrictive group 10 of 16 observations fell below the regression line for elderly subjects. HV could not be determined in two patients in group A nor in one patient in group B because of the radiological lung changes.

THb, BV and HV mean values were not significantly different in the two groups, except for a smaller BV in men in group A compared to group B ($p < 0.01$) but the difference was not significant when BV was related to body weight ($p > 0.05$) (Table III).

Comments

According to the functional classification the patients of group A had obstructive ventilatory dysfunction while group B fulfilled the criteria of restrictive ventilatory dysfunction. However the two groups had practically the same ventilatory capacity (Table VI). Group A had a significantly lower ventilatory capacity than group B was 20

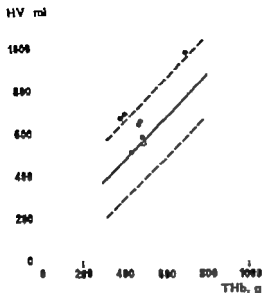


Figure 1 Heart volume (HV) in relation to total amount of hemoglobin (THb) in 16 men (solid symbols) and 6 women (open symbols) with chronic obstructive lung disease. Whole line normal regression line. Interrupted lines ± 2 SD (5 17 21)

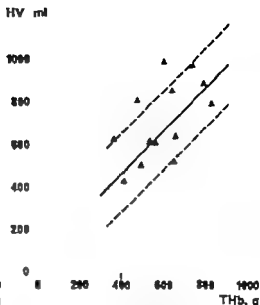


Fig. 2 Heart volume (HV) in relation to total amount of hemoglobin (THb) in 11 men (solid symbols) and 5 women (open symbols) with chronic restrictive lung disease. Whole line normal regression line. Interrupted lines ± 2 SD (5, 17 21)

The low ventilatory capacity in the restrictive group is attributed to the reduced vital capacity (see correlation matrix, Chapter VI). Theoretically these patients should be able to counteract the effect of reduced vital capacity by increasing their rate of breathing. This is possible if there are no factors limiting the speed of movement. In fact these patients have stiff lungs and thoracic cage (two patients had thoracoplasty). In the obstructive group THb and BV were reduced in men but not in women, probably because men have a higher degree of physical inactivity in relation to their normal life activity. In the restrictive group men had a reduced THb but

rather normal BV that is to say they were slightly anemic.

The mean heart volume was normal in relation to THb in both groups but showed wide individual variations.

Groups A and B were comparable in many respects, being identical in age, body size and circulatory dimensions, (THb, BV and HV) Table III

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CHAPTER III

EXERCISE TOLERANCE IN PATIENTS WITH OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

The introduction of exercise tolerance testing in clinical medicine has increased our ability to assess derangements in pulmonary and circulatory functions (11, 18). The physical working capacity (PWC) is measured in many ways, depending on the type of patient and the aim of the assessment. In patients with cardiovascular disease PWC can be determined from the heart rate-work load or the heart rate-oxygen uptake relationship, using maximal or submaximal loads, (1, 18). In patients with chronic lung disease, in whom working capacity is limited by respiration, PWC can be determined as the maximal performed work of a few minutes duration (10).

The purpose of the present investigation of patients with obstructive and restrictive lung disease was to study 1) the rate of work at the breaking point of exercise (W_B) and its relation to respiratory data, 2) the rate of work at heart rate 130 beats/min (W_{130} and W_{B_2}) and their relations to circulatory dimensions such as heart and blood volumes, 3) the variation of heart rate at the exercise breaking point (HR_B) within and between the obstructive and restrictive groups of patients.

Results

Orthostatic test. Tables I—II. In group A the heart rate (HR) at rest in supine position averaged 84 ± 15 and in group B 76 beats/min, $SD \pm 14$. In standing position HR increased significantly in both groups ($p < 0.001$) and in group A averaged 98 beats/min, $SD \pm 15$ and in group B 94 beats/min, $SD \pm 17$. The mean values at rest and the mean heart rate in standing position for each group were within normal variation, but the interindividual variations were large. Five patients in group A (Nos. 3, 4, 11, 15 and 24) and two in group B (Nos. 3 and 16) had a heart rate in supine position of ≥ 95 beats/min, in standing position ten patients in A and four in B had a marked increase in heart rate to a level > 100 beats/min; this included the patients with high initial supine heart rate. One case in group A (No. 20) showed a vasovagal reaction on standing, as revealed by a lower HR in standing than in supine position.

Exercise tolerance test. Tables I—II. In group A the W_B in sitting position averaged in men 432 kpm/min, $SD \pm 231$ (range 100—900) and in women 309 $SD \pm 121$ (range 100—400). In

Table 1. Exercise tolerance test in group A. HR = heart rate, HR_{br} = breaking point heart rate, W_{br} = rate of work at the exercise breaking point, W₃₀ = rate of work at heart rate of 130 beats/min.

Subject no	REST		EXERCISE				Cause of interruption in sitting position	
	HR	Sepsis	HR _{br} beats/min.		W _{br} kgm/min.			
			Standing	Sitting	Sitting	Sepsis		
1	109	92	175	120	565	565	300	dyspnoea — high heart rate
2	64	46	110	121	450	465	—	dyspnoea
3	108	99	170	141	600	575	250	dyspnoea
4	116	102	153	154	735	200	—	dyspnoea
5	88	70	137	140	665	600	700	dyspnoea
6	86	80	170	167	900	900	600	high heart rate
7	86	74	152	129	800	535	600	dyspnoea — fatigue in the legs
8	94	86	118	116	400	265	500	dyspnoea
9	94	80	126	120	400	335	450	dyspnoea
10	84	72	92	—	100	—	—	dyspnoea
11	116	98	138	129	315	285	200	dyspnoea
12	100	84	98	116	250	200	—	general fatigue
13	104	88	113	108	150	150	—	dyspnoea
14	104	92	147	142	235	200	200	dyspnoea — ectopic beats
15	116	104	134	104	350	400	400	dyspnoea
16	96	84	132	130	300	265	300	dyspnoea
17	100	82	156	162	400	400	250	dyspnoea — fatigue in the legs
18	114	84	132	110	275	200	—	dyspnoea — general fatigue
19	96	80	134	108	250	65	300	dyspnoea — fatigue in the legs
20	88	92	162	136	400	265	230	dyspnoea
21	70	50	132	96	400	400	400	dyspnoea
22	102	80	157	134	450	400	270	dyspnoea
23	94	84	113	114	100	100	—	dyspnoea
24	132	116	150	142	235	235	—	dyspnoea

T 44 // Exercise tolerance test in group B. Symbols as in Table I.

Subject no.	RST		EXERCISE				W ₁₂₀		Cause of interruption in sitting position
	HR		HR ₁₂₀ beats/min.		W ₆₀ kpm/min.		Sliding	Supine	
	Supine	Standing	Sitting	Supine	Sitting	Supine			
1	74	84	148	138	400	265	300	200	dyspnoea
2	72	90	128	102	400	265	400	—	dyspnoea
3	102	131	167	137	600	535	200	290	dyspnoea
4	68	80	120	132	400	400	470	400	dyspnoea
5	77	82	161	140	700	600	500	500	dyspnoea
6	85	102	140	121	465	400	450	470	dyspnoea — general fatigue
7	68	86	128	129	335	265	335	365	dyspnoea
8	58	94	131	125	400	400	400	450	ectopic beats
9	68	88	109	141	600	535	600	400	dyspnoea — cough — general fatigue
10	70	92	109	102	400	200	—	—	ST depression — ectopic beats
11	84	96	119	118	250	200	—	—	dyspnoea
12	55	72	160	137	800	700	550	450	dyspnoea — fatigue in the legs
13	66	78	170	150	635	535	400	450	dyspnoea
14	68	92	138	122	450	400	570	470	dyspnoea — fatigue in the legs
15	74	94	144	140	200	200	150	100	dyspnoea
16	100	122	140	132	500	350	150	370	dyspnoea — cough — general fatigue
17	94	118	136	135	400	200	200	200	dyspnoea

group B the average in men was 479 kpm/min, SD ± 161 (range 250—800) and in women 397 kpm/min, SD ± 164 (range 200—635). The mean values were significantly lower than values predicted from the equation given by Irnell and Linder in normal subjects (8). There was no difference of significance between the group means for either sex ($p > 0.05$). As men and women were included in one group W_B for men and women together in group A was 391 kpm/min, SD ± 207 corresponding to 52 % of the predicted value, and in group B 455 kpm/min, SD ± 162 , or 55 % of the predicted value.

In group A the W_B in sitting position was studied as a dependent variable in relation to measurements of lung function, size of the cardiovascular system and body size (Table III). The highest significant correlations were with the indices of the ventilatory capacity FVC, FEV, MVV_{10} and MVV_{75} ($p < 0.001$). The second highest correlations were with RV/TLC %, VC and W_{120} ($p < 0.01$). The correlation between W_B and MVV_{10} was higher than that with W_{120} , although only values from 16 patients in whom W_{120} could be determined were included in the analysis ($r = 0.845$ $p < 0.001$). There was a probably significant correlation to FEV %, FRC/TLC, THb, BV and weight ($p < 0.05$). No correlation was found to TLC, RV, FRC, HV, height or the respiratory rate at work loads 200 and 400 kpm/min (RR_{200} and RR_{400}). Except for RV/TLC % and FRC/TLC % all correlations were positive. The regression of W_B on FEV is shown in Fig. 1. When W_B in group B was compared

with the same independent variables listed in Table III there was a significant correlation to THb $r = 0.704$ $p < 0.01$ $n = 17$. There was a correlation of probable significance with BV ($r = 0.547$ $n = 17$) weight ($r = 0.601$ $n = 17$) W_{120} ($r = 0.609$ $n = 15$) and RR_{200} ($r = 0.591$ $n = 14$). No significant correlation was found between W_B and VC, nor was there a correlation when only eight patients with VC < 50 % of the predicted value were included. Similarly there was no correlation to any other static or dynamic lung volumes. The relationship between W_B and FEV in group II is illustrated in Fig. 2.

In group A the W_B in sitting position in men averaged 409 kpm/min, SD ± 174 $n = 11$ and in women 290 kpm/min, SD ± 67 $n = 5$. In group II the figures were for men 441 kpm/min, SD ± 163 $n = 10$ and for women 258 kpm/min, SD ± 118 $n = 5$. The mean values are significantly low compared to normal values (8). In group A the W_{120} was significantly correlated to heart volume and height ($p < 0.01$). There was a correlation of probable significance with THb, BV, weight, FVC, FEV and MVV_{10} . In group II the W_{120} was highly correlated to THb ($p < 0.001$) and significantly correlated to BV and weight ($p < 0.01$). The correlation with HV was probably significant ($p < 0.05$). The relationships with other variables were not significant.

HR_B in sitting position averaged 138 beats/min in both groups. Only three patients in group A and two in group B had a heart rate around 170 beats/min. All other patients in both groups inter

Table III Summary of correlation analysis.

Dependent variable	W _B		HR _B		W ₁₂₀	
Independent variable	A	B	A	B	A	B
TLC	—	—	—	—	—	—
VC	++	—	—	—	—	—
RV	—	—	++	—	—	—
FRC	—	—	++	—	—	—
RV/TLC %	++	—	++	—	—	—
FRC/TLC %	++	—	++	—	—	—
FVC	+++	—	+	—	+	—
FEV ₁	+++	—	++	—	+	—
FEV %	+	—	++	—	—	—
MVV ₀	+++	—	+	—	+	—
MVV _{free}	+++	—	+	—	—	—
THb	+	++	—	++	+	+++
BV	+	+	—	+	+	++
HV	—	—	—	—	++	+
Height	—	—	—	—	++	—
Weight	+	+	—	+	+	++
W ₁₂₀	++	+	—	—	—	—
RR ₂₀₀	—	+	—	—	—	—
RR ₁₀₀	—	—	—	—	—	—

+ <0.05

++ <0.01

+++ <0.001

— non-significant

rupted the test before reaching a heart rate of 170 beats/min. The cause of interruption of the test (Tables I—II) was mostly dyspnea, in a few cases fatigue in the legs or general fatigue. In group II the cause of interruption was coronary insufficiency and ectopic beats in patient 10 and ectopic beats in patient 8.

In group A low HR_B in sitting work was found in patients with high RV FRC, RV/TLC % and FRC/TLC % and low ventilatory capacity. HR was

not correlated to W₁₂₀, nor was there any correlation of significance to THb, BV, HV, height, weight, RR₂₀₀ or RR₁₀₀. In group II the HR_B was correlated to THb ($p < 0.01$), BV and weight ($p < 0.05$). No correlation was found with any of the other variables listed in Table III.

The summary of the correlation analysis as regards W_B, HR_B and W₂₀₀ is given in Table III.

The mean heart rate at work load 200

Table II Exercise tolerance test: differences between group means (A minus B) in sitting and supine position, mean values \pm SD, number of observations in parentheses.

Variable	Group A	Group B	\bar{d}	P
HR, rest-supine	84.0 \pm 15.4 (24)	75.5 \pm 13.5 (17)	8.5	—
HR, standing	98.3 \pm 15.3 (24)	93.8 \pm 16.6 (17)	4.5	—
HR ₂₀₀ sit.	111.5 \pm 18.1 (20)	107.5 \pm 16.5 (15)	4.2	—
HR ₂₀₀ sup.	112.5 \pm 18.0 (18)	111.5 \pm 14.5 (16)	1.0	—
RR ₂₀₀ sit.	24.5 \pm 4.5 (20)	24.8 \pm 7.0 (14)	— 0.3	—
RR ₂₀₀ sup.	24.8 \pm 4.5 (16)	27.2 \pm 9.5 (12)	— 2.4	—
HR ₄₀₀ sit.	131.1 \pm 21.6 (12)	125.3 \pm 15.9 (12)	5.8	—
HR ₄₀₀ sup.	121.6 \pm 21.6 (7)	128.4 \pm 7.5 (8)	— 6.8	—
RR ₄₀₀ sit.	27.3 \pm 7.5 (11)	28.6 \pm 5.7 (12)	— 1.3	—
RR ₄₀₀ sup.	29.4 \pm 7.5 (9)	28.9 \pm 6.9 (8)	— 0.5	—
W ₂₀₀ sit.	371.9 \pm 157.2 (16)	379.7 \pm 169.9 (15)	— 7.8	—
W ₂₀₀ sup.	337.1 \pm 160.2 (12)	354.6 \pm 119.6 (14)	— 17.5	—
W ₀ sit.	391.0 \pm 207.1 (24)	435.0 \pm 161.5 (17)	— 44	—
W ₀ sup.	335.0 \pm 183.9 (23)	379.0 \pm 157.1 (17)	— 44	—
HR ₀ sit.	137.5 \pm 22.6 (24)	138.1 \pm 18.8 (17)	— 0.6	—
HR ₀ sup.	127.7 \pm 18.6 (23)	131.7 \pm 16.0 (17)	— 4.0	—

and 400 kpm/min HR₂₀₀, HR₄₀₀, (Table IV) was in both groups higher than in normal subjects (5)

The respiratory rate at work load 200

and 400 kpm/min RR₂₀₀, RR₄₀₀, (Table IV) was in both groups higher than reported in normal subjects by Grimby and Söderholm (5). The inter

Table 1 The effect of body position on the measurements during exercise tolerance tests, analysed as difference between the mean values (sitting minus supine)

Variable	Group A					Group B				
	\bar{d}	SD _d	C	n	P	\bar{d}	SD _d	C	n	P
HR, rest, beats/min. (supine — standing)	—14	6.3	4.9	24	**	—18.4	7.7	6.4	17	***
HR ₃₀₀ beats/min.	—1.8	15.3	9.6	18	—	—4.4	11.8	7.6	15	—
RR ₃₀₀ cpm	—1.4	3.1	9.1	16	—	—2.1	3.6	9.8	12	—
HR ₄₀₀ beats/min.	4.4	13.6	8.9	7	—	—5.5	15.9	8.6	8	—
RR ₄₀₀ cpm	—2.7	4.7	11.7	9	—	—0.3	3.9	9.5	8	—
W ₁₂₀ kpm/min.	—1.9	102	20.4	11	—	23.6	138.7	26.8	14	—
W ₃₀ kpm/min.	69	78	14.9	23	***	76	68.2	11.6	17	***
HR ₃₀ beats/min.	12	17.9	9.2	23	**	6.4	13.7	7.1	17	borderline significance

\bar{d} = mean difference; SD_d = standard deviation of the difference; C = coefficient of variation; n = number of observations; P = level of significance

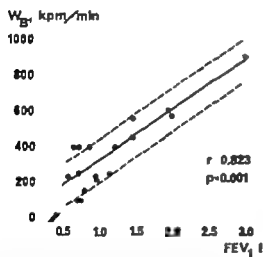


Fig 1 Relationship between W_B and forced expiratory volume in one second (FEV_1) in group A, 24 patients with obstructive lung disease. Regression line ± 1 SD $Y = 39.2 + 262.5 \times$ SD 120.

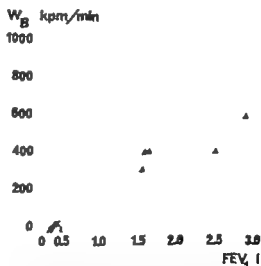


Fig 2 Scatter diagram illustrating the relationship between W_B and FEV_1 in group B, 17 patients with restrictive lung disease.

Individual variation was also large at 200 lpm/min the range in group A was 17–32 cpm, $n = 20$ and in group B 16–39 cpm, $n = 14$. At 400 lpm/min the range in group A was 18–44 cpm, $n = 11$ and in group B 18–37 cpm, $n = 12$.

In group A the RR_{200} and RR_{400} were not correlated to VC, FEV_1 or MVV_{40} , while in group B there was a negative correlation of probable significance between RR_{200} and VC ($r = -0.652$) FEV_1 ($r = -0.616$) and MVV_{40} ($r = -0.547$ $n = 14$). No correlation was found between RR_{400} and those three variables.

The effect of body position

In standing position the mean heart rate was significantly higher than in supine position in both groups ($p < 0.01$). W_B was significantly higher in sitting than in supine position in both groups ($p < 0.001$). The mean difference was 69 lpm/min in group A and 76 lpm/min in group B. The mean HR_B was also higher in sitting position than in supine position, the mean difference in A being 12 beats/min ($p < 0.01$) and in B 6 beats/min (borderline significance). There was no difference of significance between mean values in sitting and supine position as regards HR_{200} , HR_{400} , W_{20} , RR_{200} or RR_{400} (Table V).

Comparing the measurements from the exercise tests in both groups, no significant differences between the group means were found (Table IV).

Discussion

According to the functional classification the patients of group A had an ob-

structive ventilatory impairment, while those of group B fulfilled the criteria of restrictive ventilatory impairment. The two groups were comparable in many respects — age, body size and dimensions of cardiovascular system — as evidenced by the absence of significant differences between the group means (Chapter II). They had practically the same ventilatory capacity and were also similar in their ability for exercise. The three exercise parameters W_B , W_{130} and HR_B were all decreased in both groups, with no significant differences between A and B.

W_B . The maximal work performance in a type of exercise as used in this study is dependent on the oxygen transporting capacity. In the obstructive group the low W_B was highly related to the low indices of the ventilatory capacity — FVC, FEV_1 , MVV_{40} and MVV_{free} . W_B was better correlated to MVV_{40} than MVV_{free} , because the first is more reproducible. The higher correlation between W_B and FEV than with FEV % is due to the fact that the latter is probably a qualitative rather than quantitative index of obstruction (16).

The positive relationship between W_B and W_{20} ($p < 0.01$) the latter being a relative measure of the stroke volume (20) indirectly signifies an association between W_B and the stroke volume, and consequently the low values of W_B found in these patients should be partly attributed to a small stroke volume. However the correlation between W_B and W_{130} was of a lower magnitude and significance compared to the correlation between W_B and the indices of ventilatory capacity which might indicate that the

stroke volume was a less important limiting factor than the ventilatory capacity. Another reason is that W_B represents subjective maximal work, which does not coincide with the maximal work estimated by extrapolation from submaximal heart rate. Bouhuys and Pool (3) showed, in patients with chronic lung disease whose work performance was limited by the ventilatory reserves, that the oxygen uptake at subjective maximal work is lower than the maximal oxygen uptake determined by extrapolation from submaximal heart rate.

The slight correlation between W_B and THb BV and the absence of correlation with HV show that the dimensions of the cardiovascular system are of minor or no importance for the limitation of W_B in these patients. The findings in this study are in agreement with those of Jones *et al* (9) who found that effort tolerance in patients with chronic airway obstruction was largely limited by a low ventilatory capacity. Also Irnell (7) found a significant correlation between the maximal performed work and the ventilatory capacity in asthmatic patients. A similar relation was found in patients with chronic bronchitis by Simonsson *et al*. (17) The results differ however from those found in healthy elderly people, in whom no significant correlation was found between maximal performed work and the functional capacity of the lungs or the circulatory dimensions (4, 21).

In contrast to the findings in the obstructive group, W_B in the restrictive group was not related to any of the static or dynamic lung volumes. However there was a positive relationship between

W_B and THb BV weight and W_{150} . Consequently the low W_B in these patients might be ascribed to the reduced circulatory dimensions. But it is rather difficult to attribute the low W_B to the small circulatory dimensions alone especially HR_B was rather far from being maximal. Also the weak correlation with BV and W_{150} , and the absence of correlation with the heart volume, may suggest the coexistence of other limiting factors.

W_{150} The physical working capacity determined as the rate of work at heart rate 170 beats/min (W_{170}) is an estimate of the oxygen pulse and is dependent on the stroke volume and arteriovenous oxygen difference. In patients with severe degree of disability extrapolation to a lower heart rate than 170 has been used, i.e. heart rate 160, 150 or as in this study 130 and the rate of work at this heart rate (W_{130}) can be used as relative measure of the stroke volume. In group A W_{130} was related to HV, THb BV and body size, which is in agreement with the findings in normal subjects (21). However the relationship with THb and BV was only of probable significance, which can partly be explained by the absence of steady-state conditions in some patients when W_{130} was determined. The slight relationship between W_{130} and FVC, FEV_1 and MVV_{40} suggests the probable importance of these parameters for the filling and emptying conditions of the heart. In group B W_{130} , as in normal subjects, was significantly related to the measurements of the cardiovascular system but was not related to the measurements of lung functions. This shows that the low

W_{120} was not attributable to the direct effect of restrictive ventilatory dysfunction.

HR_B. In normal subjects the physical working capacity is determined mainly by the stroke volume (2, 20). In patients with low PWC and having a small stroke volume the cardiac output is secured by increasing the heart rate to maximal level, which is reached at low work loads. In groups A and B the HR_B did not reach maximal levels except in a few cases, which indicates that the cardiovascular system was not stressed to maximal level. In the *obstructive group* HR_B in the same way as W_B was related to the measurements of the ventilatory capacity. It was, moreover, negatively correlated to RV/FRC, RV/TLC % and FRC/TLC % thus reflecting the relationship between the high degree of obstruction and the early interruption of exercise. In the *restrictive group* the relationship between HR_B and THb BV and the absence of correlation with HV static and dynamic lung volumes, show the parallelism between HR_B and W_B .

The higher than normal heart rate at submaximal work loads (HR₂₀₀, HR₄₀₀) in the two groups signifies a smaller than normal stroke volume at these work loads.

The respiratory rate at submaximal work load was not related to the degree of airway obstruction. A similar finding has been reported by others (17). In the restrictive group a slight negative correlation was found between the vital capacity and the respiratory rate at 200 kpm/min. This may be explained by the patients' attempt to counteract the effect of reduced vital capacity by in-

creasing their respiratory rate. However the relationship was not significant at higher level of ventilation (400 kpm/min) probably due to the involvement of other factors affecting the work of breathing which force the patients to choose their own optimal frequency of breathing (6, 13). The latter mechanism might explain the large variation in respiratory rates in the two groups.

The effect of body position. The lower W_B and HR_B in supine position than in sitting position accord with the findings in elderly healthy men by Strandell (21) who attributed the difference to the effect of hydrostatic counterpressure and to untrained, or a smaller engagement of muscle groups in supine exercise. Other contributory factors may be of importance, e.g. the reduction in lung volumes in recumbent position (12, 22) irrespective of its mechanism (19, 23) causes a decrease in the cross-sectional area of the airways. Consequently the work of breathing becomes higher in supine position because of an increased airway resistance (14, 15). These factors explain the lower work performance in supine than in sitting position.

The change in body position did not have a significant effect on W_{120} and heart rates at submaximal work loads, indicating a minimal orthostatic effect during exercise. Similarly there was no effect on the respiratory rates.

Summary

1. W_B , W_{120} and HR_B were reduced in both the obstructive (A) and restrictive (B) groups.

- 2 The cause of interruption of the exercise test was dyspnea in 96 % of the patients in group A and 88 % of the patients in group B
- 3 W_B in group A was highly correlated to the ventilatory capacity ($p < 0.001$) and negatively correlated to RV/TLC % and FRG/TLC % ($p < 0.01$). It was weakly related to the circulatory dimensions. W_B in group B was not related either to the lung volumes or to ventilatory capacity but significantly correlated to THb ($p < 0.01$) and BV ($p < 0.05$)
- 4 In group A low HR_B was found in patients with low ventilatory capacity and large lung volumes, while in group B it was associated with reduced THb and BV
- 5 W_{130} in group A was correlated, though weakly with the ventilatory capacity and circulatory dimensions, while in group B it was significantly correlated with the circulatory dimensions.
- 6 W_B and HR_B were significantly higher in sitting than in supine position in both groups, while W_{130} as well as the heart and respiratory rates at submaximal work were not significantly different in the two positions.
- 7 The respiratory rate at submaximal work showed wide variations in the two groups. In group A it was not related to the degree of obstruction while in group B RR_{sub} was negatively but weakly correlated with the vital capacity

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CHAPTER IV

THE CAUSES OF HYPOXEMIA IN PATIENTS WITH OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

Arterial hypoxemia is a common feature in chronic obstructive and restrictive lung disease. Patients with chronic interstitial fibrosis and similar clinical conditions often exhibit severe arterial hypoxemia during exercise despite a high alveolar ventilation (4-5). Some investigators (4-30-34) have studied the cause of hypoxemia during exercise in patients with pulmonary fibrosis, but its mechanism has not been fully elucidated. The present study was undertaken in order to evaluate the factors contributing to hypoxemia, particularly during exercise, in patients with obstructive and restrictive lung disease.

Procedure

The examinations were carried out early in the morning after a light breakfast. No smoking was allowed after 7 o'clock in the evening before examination. A catheter was introduced usually in the left brachial artery by a percutaneous Seldinger technique; this was followed by determination of ventilation and alveolar gas exchange at rest in sitting position. After a resting period of about 15 minutes the diffusing capacity was determined during steady-state exercise using 0.05 % carbon monoxide in air.

The expired air was collected for 3 minutes in a Douglas bag. In the middle of this period two arterial blood samples were withdrawn for determination of gas tensions, pH and carbon monoxide content. The work period was as a rule 7 minutes. After a resting period of 30 minutes the nitrogen wash-out time and the veno-arterial shunt were measured. Blood samples for determination of $P_{a_{O_2}}$ and $P_{a_{CO_2}}$ were withdrawn at 10 minutes and at the end of the breathing period and analysed immediately within half a minute. The sample with the highest $P_{a_{O_2}}$ was used in the calculation.

Results

Respiratory data are shown in Tables I—II.

In group B no significant difference was found between patients with fibrotic restriction and patients with pleural restriction, either at rest or during exercise, as regards the following variables: V_D/V_T , $P_{A_{O_2}}$, $P_{A_{CO_2}}$, $P(A-a)_{O_2}$ and $\dot{D}_{L_{CO}}$. Therefore they were treated as one group.

The oxygen uptake (\dot{V}_{O_2}) at rest and during exercise was similar in both groups, $p > 0.05$.

Total ventilation (V_E) at rest was

Table 1 Respiratory data at rest and during increasing exercise in group A. 13 patients with chronic obstructive lung disease.

W	\dot{V}_O_2 ml/min	\dot{V}_E l/min	\dot{V}_E/\dot{V}_O_2	\dot{V}_T ml	Resp. ml cycle	V_D ml	V_D/V_T	
Rest	\bar{V}	287	11.4	40.6	609	20	289	0.48
	range	203-460	8.3-14.9	25.1-56.0	281-863	12-32	81-446	0.30-0.60
Exercise	\bar{V}	839	23.0	51.2	1035	26	375	0.37
	range	406-1464	14.2-31.4	19.1-164	477-1803	15-40	186-686	0.19-0.61
	\dot{V}_A l/min	P_{aO_2} mm Hg	P_{aO_2} mm Hg	P_{aCO_2} mm Hg	pH	R	$P_{aH_2O_2}$ mm Hg	\dot{D}_{tO_2} ml/min/ mm Hg
Rest	\bar{V}	5.2	102	67	40	7.41	0.82	35
	range	3.7-7.5	86-115	48-77	30-57	7.35-7.51	0.64-0.95	0-54
Exercise	\bar{V}	14.6	98	64	44	7.35	0.83	35
	range	5.4-29.1	76-111	39-89	33-71	7.28-7.50	0.70-0.97	20-58
								17.5
								7.0-33.2

Table II Respiratory data at rest and during sitting exercise in group III 17 patients with bronchiectatic lung disease

	\dot{V}_E l/min/min	\dot{V}_{O_2} ml/min	\dot{V}_L l/min	\dot{V}_E/\dot{V}_{O_2}	\dot{V}_T ml	Resp. rate cycles	\dot{V}_D ml	\dot{V}_D/\dot{V}_T
Rest	$\bar{\quad}$ range	290 209—311	11.0 7.6—14.9	38.4 26.7—54.0	568 355—872	20 10—8	27 117—363	0.41 0.20—0.57
Exercise	$\bar{\quad}$ 81 100—500 range	968 610—1471	29.6 17.7—43.7	50.7 18.1—38.8	1173 587—1722	27 17—39	370 136—634	0.32 0.11—0.50
Rest	$\bar{\quad}$ range	\dot{V}_A l/min	P_{aO_2} mm Hg	P_{aCO_2} mm Hg	pH units	R	$P_{(A-a)O_2}$ mm Hg	D_{LCO} ml/min/mm Hg
		3.7 4.0—8.8	74 56—90	36 7—44	7.43 7.36—7.50	0.80	32 18—48	
Exercise	$\bar{\quad}$ range	18.6 9.6—26.6	67 5—88	40 29—53	7.38 7.32—7.43	0.86	38 3—50	17.1 8.6—29.8

n = 16

high in relation to oxygen uptake in both groups and increased during exercise due to an increase both in respiratory rate and in tidal volume. No significant difference was found between the group means, $p > 0.05$

Alveolar ventilation (V_A) at rest was of the same order in both groups. During exercise it was slightly higher in group B, $p < 0.05$. The alveolar ventilation increased with increasing V_{O_2} in group A according to the equation $V_A = -1.33 + 0.019 V_{O_2}$ ($r = 0.917$, $SD \pm 2.68$, $n = 24$, $p < 0.001$) and in group B according to the equation $V_A = 4.97 + 0.014 V_{O_2}$ ($r = 0.782$, $SD \pm 3.21$, $n = 16$, $p < 0.001$). Thus at a given oxygen uptake V_A during exercise was higher in group B than in group A.

The arterial carbon dioxide tension (P_{aCO_2}) averaged in group A at rest 40 mm Hg and during exercise 44 mm Hg. Considering the normal P_{aCO_2} range to be between 35–45 mm Hg, 4 patients had moderate to severe elevation of P_{aCO_2} at rest and during exercise and 3 a slight elevation only during exercise. In group B P_{aCO_2} averaged at rest 36 mm Hg and during exercise 40 mm Hg. One patient had a moderate elevation of P_{aCO_2} during exercise. In both groups a few patients showed a lower level of P_{aCO_2} . During exercise P_{aCO_2} increased 4 mm Hg in both groups.

The physiological dead space (V_D) and the *dead space ventilation ratio* (V_D/V_T) were higher at rest than values reported in healthy subjects (20–24–32). V_D/V_T averaged 0.48 in group A and 0.41 in group B. During exercise V_D in-

creased in both groups due to an increase in V_T and respiratory rate. However the increase in V_T exceeded the increase in V_D , so that the ratio V_D/V_T decreased 9% in both groups, the mean value in group A being 0.39 and in group B 0.32. The difference between the two groups at rest and during exercise was of probable significance $p < 0.05$.

Alveolar oxygen tension ($P_{A_{O_2}}$) in group A averaged at rest 102 mm Hg and decreased slightly during exercise. In group B the average was 106 mm Hg at rest and was essentially unchanged during exercise.

The arterial oxygen tension (P_{aO_2}) was slightly lower in group A than in group B, $p < 0.05$ and averaged 67 mm Hg range 48–77 in group A and 74 mm Hg range 56–90 in group B. The lowest values predicted (mean -2 SD) according to the equation given by Mellemgaard (20) taking into account the decrease of P_{aO_2} with age, was 76 mm Hg in group A and 77 mm Hg in group B. During exercise the decrease in P_{aO_2} in group A (with a higher V_D/V_T ratio) was not significant ($p > 0.05$) and was of lower magnitude, 3 mm Hg ($p > 0.05$) than in group B (with a lower V_D/V_T) which showed a fall of P_{aO_2} 7 mm Hg ($p < 0.05$).

The alveolar arterial oxygen tension difference $P(A-a)_{O_2}$ was at rest large in both groups compared to normal values (20–24–32). In group A it was 35 mm Hg range 0–54 and was essentially unchanged during exercise. In group B the average at rest was 32 mm Hg, range 18–48 and during exercise 38 mm Hg range 23–50 an increase of

6 mm Hg ($p < 0.05$) 11/24 patients in group A and 5/16 patients in group B showed a decrease or an unchanged $P(A-a)_{O_2}$ difference during exercise.

Diffusing capacity of the lungs ($D_{L_{CO}}$) was determined during sitting exercise in 22 patients in group A, of whom one was discarded, and in 16 patients in group B. Two patients in group A and one in group B had to interrupt the work shortly after the start. In group A the mean work load was 245 kpm/min, range 100–600 corresponding to 63 % of the rate of work at the breaking point of exercise (W_B) obtained during the exercise tolerance test. The mean heart rate was 121 beats/min, range 98–155 corresponding to 88 % of the heart rate at the breaking point of exercise (HR_B). In group B the mean work load was 284 kpm/min, range 100–500 corresponding to 62 % of W_B , and the mean heart rate 123 beats/min, range 110–148, corresponding to 89 % of HR_B . $D_{L_{CO}}$ was identical in the two groups and in group A averaged 17.3 ml/min/mm Hg, range 7.0–33.2, and in group B 17.1 ml/min/mm Hg, range 8.6–29.8.

The individual $D_{L_{CO}}$ values in each group were compared to normal values predicted from the equation given by *Donevan et al* (10) using the age and the oxygen uptake as independent variables. In group A nine patients had $D_{L_{CO}}$ lower than the predicted values, nine had higher values, while three had values equal to predicted values (Fig. 1). In group B nine patients had a reduced $D_{L_{CO}}$ four had higher values and three had values equal to predicted values

(Fig. 2). In this material some patients were above 50 years of age, which was given as the highest age in *Donevan's* material. When only patients aged 50 years or below were included, the mean difference between observed and predicted values was –5.2 units in group A ($p > 0.05$) $n = 6$) and –6.3 units in group B ($p < 0.05$) $n = 9$). The change of $D_{L_{CO}}$ with V_{O_2} in group A was in accordance with the equation $D_{L_{CO}} = 1.600 + 0.022 V_{O_2}$ and in group B with the equation $D_{L_{CO}} = -2.950 + 0.020 V_{O_2}$. Neither the slope nor the intercept were significantly different in the two groups ($p > 0.05$).

In group A there was a significant correlation between $D_{L_{CO}}$ and both $\dot{V}_{V_{max}}$ and FEV_1 ($p < 0.01$) and a probably significant correlation with THb and BV ($p < 0.05$) but no significant correlation with TLC, VC, RV and FRC. In group B the $D_{L_{CO}}$ was significantly correlated to THb ($r = 0.686$, $p < 0.01$) and BSA ($r = 0.659$, $p < 0.01$). There was no significant correlation to the spirometric data. Nor was there a correlation between $D_{L_{CO}}$ and VC in patients with fibrotic restriction or pleural restriction.

The *pulmo-arterial* shunt in group A averaged at rest 10.7 % of the cardiac output, range 6.3–18 and in group B 8.7 % range 4.3–13 the difference between the group means not being significant. The mean values were higher than those found in healthy subjects (33).

A further analysis of the results was made by selecting some patients from group B with the greatest fall of Pa_{O_2} ,

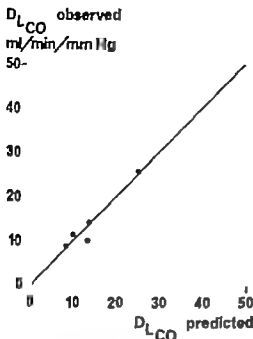


Fig 1 D_{LCO} during exercise observed in 14 men (solid symbols) and 7 women (open symbols) with chronic obstructive lung disease in relation to predicted values. Solid line = identity line.

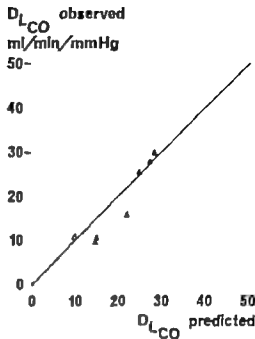


Fig 2 D_{LCO} during exercise observed in 11 men (solid symbols) and 5 women (open symbols) with chronic restrictive lung disease. Solid line = identity line.

during exercise. Six patients were selected (Table III) four of whom had fibrotic restriction (Nos. 1, 2, 5 and 6) and two pleural restriction (Nos. 3 and 4). The fall of P_{aO_2} during exercise in these patients varied between 14–25 mm Hg and the corresponding increase in $P(A-a)_{O_2}$ was 7–25 mm Hg. During exercise 1/6 had a normal V_D/V_T ratio and 4/6 had $P_{aO_2} \geq 103$ mm Hg. D_{LCO} was normal in relation to age in two patients, moderately reduced in another two, and severely impaired in the remaining two patients. In these patients the overall V_A/\dot{Q} ratio and the diffusing capacity/perfusion ratio, D_{LCO}/Q ,

for the whole lung were calculated for each patient during exercise. As the flow (\dot{Q}) was not measured during this examination, \dot{Q} -values were calculated using the regression equation for cardiac output on oxygen uptake obtained during hemodynamic study of the patients in this material. It is necessary to point out that the calculated Q -values correspond to values measured in the supine position, while the oxygen uptake in this study was measured in sitting position, which would introduce an error. However these values are used solely for comparative purposes. The magnitude of the fall in the arterial oxygen tension during

TABLE III Ventilatory data in 6 selected patients with restrictive lung disease at rest and during exercise.

		V_D/V_T	P_{AO_2} mm Hg	P_{aO_2} mm Hg	P_{aCO_2} mm Hg	$P(A-a)O_2$ mm Hg	DL_{CO} ml	V_A/\dot{Q}	DL_{CO}/\dot{Q}	ΔP_{aO_2} mm Hg
1	S.H.	0.42	117	90	24	27				
		0.38	107	65	38	42	12.2	1.68	1.02	25
2	V.S.	0.35	100	77	42	23				
		0.50	103	55	47	48	9.9	1.47	0.97	22
3	C.J.	0.42	102	84	41	18				
		0.77	106	64	44	42	16.0	1.65	1.24	20
4	E.O.	0.44	105	77	37	28				
		0.39	96	61	46	35	16.0	1.50	1.59	16
5	E.P.	0.33	99	79	44	20				
		0.1	93	64	47	29	28.1	1.48	1.85	15
6	J.S.	0.43	107	75	36	32				
		0.90	110	61	40	49	29.8	1.85	2.19	14

TABLE IV Comparison between group A and group B ventilatory data including calculated V_A/\dot{Q} and DL_{CO}/\dot{Q} at rest (R) and during exercise (E)

		V_D/V_T	P_{AO_2}	P_{aO_2}	$P(A-a)O_2$	ΔP_{aO_2}	$\Delta P(A-a)O_2$	V_A/\dot{Q}	DL_{CO}/\dot{Q}
A	R	0.48	102	67	35			0.6	—
	E	0.39	99	64	35	—3	0	1.5	1.8
B	R	0.41	106	74	32			0.7	—
	E	0.32	105		38	—7	6	1.7	1.5

also obtained during heart-thorax scan

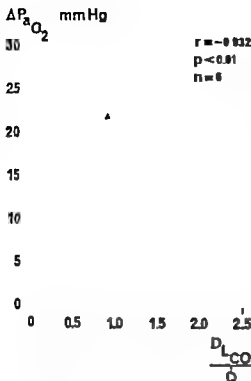


Fig 3 Difference between P_{aO_2} at rest and during exercise (ΔP_{aO_2}) in relation to the diffusing capacity/perfusion ratio in six selected patients with restrictive lung disease

exercise (ΔP_{aO_2}) showed a high negative correlation with D_{LCO}/Q , $r = -0.932$, $p < 0.01$ (Fig 3) but no significant correlation was found with \dot{V}_A/\dot{Q} or V_D/V_T , $p > 0.05$. The mean \dot{V}_A/\dot{Q} and D_{LCO}/Q during exercise were then calculated in the same way in groups A and II. The \dot{V}_A/\dot{Q} ratio was found to be lower in the obstructive group and averaged 1.5 compared to 1.7 in the restrictive group and conversely the D_{LCO}/Q ratio was higher in the obstructive group (1.8) than in the restrictive group (1.5) (Table IV).

Discussion

The point of departure for this discussion is the observation of a significant fall in arterial oxygen tension and increase in $P(A-a)_{O_2}$ difference during exercise in the restrictive group despite a high level of alveolar ventilation, in contrast to a minor fall in P_{aO_2} and unchanged $P(A-a)_{O_2}$ in the presence of low ventilation in the obstructive group.

At rest maldistribution of ventilation in relation to perfusion was evidenced in both groups by high V_D/V_T and V_D . This finding is in agreement with previous studies in obstructive lung disease (1, 8, 16, 21) in pulmonary fibrosis (1, 12, 25, 30, 34) also in patients with pleural restriction (18) and with pulmonary tuberculosis treated with pneumothorax (2). The veno-arterial shunt was increased in both groups. If one assumes that the contribution of the diffusion component to the $P(A-a)_{O_2}$ gradient at rest is negligible (26) then the low level of P_{aO_2} and large $P(A-a)_{O_2}$ difference in groups A and II are mainly attributable to inequality of ventilation-perfusion relationship and to increased veno-arterial shunt. These changes were more pronounced in the obstructive group.

During exercise there was an improvement in the distribution of the ventilation in relation to perfusion in the two groups, as evidenced by the lower V_D/V_T . However the derangements in the restrictive group were less marked than in the obstructive group, as shown by V_D/V_T and the high level of alveolar ventilation, as also by the absolute value and in relation to the oxygen uptake.

Nonetheless the fall in the arterial oxygen tension was more pronounced in the restrictive group. Most studies on the mechanism responsible for hypoxemia in restrictive lung disease have been carried out in patients with pulmonary fibrosis (alveolar capillary block) and been based on the analysis of three classical factors contributing to $P(A-a)_{O_2}$ difference, namely 1) inequality of ventilation-perfusion, 2) venous admixture, 3) diffusion limitation. It has been suggested (34) that hypoxemia during exercise is caused to a large extent by ventilation-perfusion inequalities. Such a change might be caused by a low compliance of the fibrotic alveoli (35) which also leads to uneven ventilation (11). This explanation is based on the increase of ventilation-perfusion inequalities during exercise in patients with fibrotic lungs. In group B however the mean \dot{V}_D/\dot{V}_T was significantly lower both at rest and exercise, than the corresponding values in the obstructive group. Staněk *et al.* (30) suggested that hypoxemia during exercise in pulmonary fibrosis is caused by the combined effect of a lowering of the oxygen saturation of the venous blood and hypoventilation of some areas. However the hemodynamic study of these patients (Chapter V) showed that, at a particular level of oxygen uptake, the mixed venous oxygen saturation was higher in the restrictive than in the obstructive group. Also the ventilatory data (Tables I—II) showed that hypoventilation was prevalent in patients with obstructive lung disease.

It has been suggested (11) that in patients with alveolar capillary block there is an increase of the veno-arterial shunt

at rest. This is caused by complete obliteration of the alveoli by the fibrotic process or by the shunting effect of severe impairment of diffusion (29). The behaviour of this shunt during exercise is not clear. There is a probability that true shunting contributes to hypoxemia during exercise in alveolar capillary block syndrome (34). However Holmgren and Svanborg (13) showed that in patients with sarcoidosis the shunt is practically constant or decreases during exercise. During oxygen breathing at rest the shunt was lower in the restrictive group than in the obstructive group, but whether this relationship remains unchanged during exercise is difficult to determine.

Impairment of diffusing capacity has been demonstrated in chronic obstructive lung disease (9, 17) in pulmonary fibrosis (4, 6, 9, 13) and in pleural restriction (19). It has been suggested that hypoxemia in pulmonary fibrosis is caused by impairment of diffusing capacity due to thickening of alveolar membrane (4, 6, 9, 13) or to its shunting effect (29). Impairment of diffusing capacity may be caused by an inability to expand the pulmonary vascular bed (7) in pleural restriction. The mean D_{LCO} was identical in groups A and B which were also similar in their anthropometric measurements (Chapter II). In the restrictive group the oxygen uptake as well as the alveolar ventilation, tidal volume and respiratory rate were slightly higher than in the obstructive group, and the mean age was slightly lower. Factors which should have contributed to a higher D_{LCO} in group B (3, 10, 27). It emerges that the diffu-

ing capacity at a given oxygen uptake was actually lower in the restrictive than in the obstructive group. This is also evidenced by the regression equation of $D_{L_{CO}}$ on V_{O_2} . A decrease in $D_{L_{CO}}$ causes an increase in the alveolar capillary oxygen tension difference. The latter averaged 42 mm Hg (range 26—71) in group A and 47 mm Hg (range 31—71) in group B. These values were far lower than those reported at maximal oxygen uptake (14).

The data for the six selected patients (Table III) showed that the lowering of $P_{a_{O_2}}$ during exercise cannot be totally attributed to the decreased diffusing capacity per se for example patients E.P. and J.S. had normal $D_{L_{CO}}$ values, none theless the changes of $P_{a_{O_2}}$ and $P(A-a)_{O_2}$ during exercise were remarkable. From the data in Table III the following observations could be made: 1) Severe lowering of $P_{a_{O_2}}$ during exercise occurred both in fibrosis and pleural restrictive conditions. 2) It was not always associated with high V_D/V_T but occurred in the presence of normal V_D/V_T . 3) It is not dependent on the level of the alveolar capillary oxygen tension difference per se.

Although some investigators have found that impairment of $D_{L_{CO}}$ in pulmonary fibrosis was caused by thickening of alveolar membrane (6-9) yet Roughton and Forster (28) have shown that the resistance of the alveolar membrane is only half of the total resistance and concluded that the resistance of the capillary blood to diffusion is of equal importance. Also Staub (31) found that most of the diffusing resistance lies in

the capillary blood and not in the alveolar membrane. In this material the low $D_{L_{CO}}$ in the restrictive group was significantly correlated with THb but no correlation was found with the spirometric measurements, which might indicate that the low $D_{L_{CO}}$ was related to derangements on the vascular side and not to ventilatory dysfunction. Staub (31) found that the regional variations in the capillary transit time have a more marked effect on lowering of the oxygen tension than has the membrane variation.

A decrease in capillary transit time causes a decrease in diffusing capacity/perfusion ratio ($D_{L_{CO}}/Q$). Piiper (22) in his theoretical analysis, showed that the $P(A-a)_{O_2}$ difference is dependent on the regional $D_{L_{CO}}/Q$ ratio rather than on the overall diffusing capacity as such. From the relationship between the oxygen uptake and diffusing capacity $D_{L_{CO}}$, on the one hand, and the oxygen uptake and flow \dot{Q} (Fick principle) on the other the relationship between the diffusing capacity and perfusion is expressed by the following equation

$$\frac{D_{L_{CO}}}{Q} = \frac{C_{a_{O_2}} - C_{\bar{v}O_2}}{P_{a_{O_2}} - P_{\bar{v}O_2}}$$

Thus at a given $P_{a_{O_2}}$ and a given mixed venous blood the oxygen content and partial pressure in the pulmonary capillary and consequently in the arterial blood, are determined by the ratio $D_{L_{CO}}/Q$. In the six selected patients (Table III) the fall in arterial oxygen tension during exercise ($\Delta P_{a_{O_2}}$) was closely related to the overall $D_{L_{CO}}/\dot{Q}$

values, $p < 0.01$ (Fig 3) but no significant correlation was found with the corresponding values of V_A/\dot{Q} . The mean V_A/Q and $D_{L_{CO}}/Q$ ratios in the two groups showed a contrasting result, namely a low V_A/Q and a high $D_{L_{CO}}/Q$ in the obstructive group while a high V_A/Q and a low $D_{L_{CO}}/Q$ were found in the restrictive group (Table IV).

Read and Williams (25) suggested that ventilation/perfusion inequality in the presence of diffusion defect, contributes to hypoxemia during exercise in patients with pulmonary fibrosis. However the implication of such a mechanism in this study should cause a more marked $P_{a_{O_2}}$ fall in the obstructive than in the restrictive group. It is apparent, therefore, that the discrepancy in the variation of $P_{a_{O_2}}$ and $P(A-a)_{O_2}$ between the two groups could be attributed to the low V_A/Q in the obstructive group and low $D_{L_{CO}}/Q$ in the restrictive group. It seems likely that the contribution of low $D_{L_{CO}}/Q$ to the magnitude of the fall in oxygen tension ($\Delta P_{a_{O_2}}$) and to the increase in $P(A-a)_{O_2}$ is more marked than the effect of the variation in V_A/Q when the diffusion defect is identical. Thus the change in level of $P_{a_{O_2}}$ and $P(A-a)_{O_2}$ seemed to depend on the interrelation between V_A/Q and $D_{L_{CO}}/Q$, i.e. on which of them predominated.

In conclusion, it may be suggested that the changes in arterial oxygen tension and the $P(A-a)_{O_2}$ difference during exercise in the obstructive and restrictive groups are explained on the

basis of uneven ventilation/perfusion and diffusion/perfusion inequalities and not on the basis of the three classical factors of ventilation/perfusion, diffusion limitation alone or venous admixture, which is in agreement with the concept of Papper (23).

Summary

- 1 In the obstructive group (A) the average $P_{a_{CO_2}}$ was normal at rest and somewhat elevated during exercise. $P_{a_{O_2}}$ was slightly lower than normal but did not fall significantly during exercise in most of the patients.
- 2 In the restrictive group (B) $P_{a_{CO_2}}$ was somewhat lower than in group A, both at rest and during exercise, and arterial P_{O_2} was somewhat higher at rest but fell markedly in some patients during exercise.
- 3 $P(A-a)_{O_2}$ was increased in both groups at rest, while during exercise it remained unchanged in group A but increased in group B.
- 4 V_D , V_D/V_T and Q_{sh}/Q showed high values in both groups, higher in A than in B.
- 5 $D_{L_{CO}}$ was slightly to severely reduced in 9/21 patients in group A and 9/16 patients in group B. It averaged 17.3 ml/min/mm Hg in group A and 17.1 ml/min/mm Hg in group B.
- 6 During exercise $P_{a_{O_2}}$ was lower in the restrictive than in the obstructive group.

- 7 On the basis of these findings it was concluded that hypoxemia during exercise was due mainly to uneven ventilation/perfusion in the obstructive group and uneven diffusion/perfusion in the restrictive group.

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CHAPTER V

CENTRAL HEMODYNAMICS IN PATIENTS WITH OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

One feature of chronic lung disease is that it may cause structural or functional changes in the pulmonary vascular bed, leading to pulmonary hypertension and subsequently right ventricular hypertrophy (*cor pulmonale*) (46). In the definition of *cor pulmonale* (46) it is specially pointed out that cases with disease of the left heart should be excluded as the increase in pulmonary artery wedge pressure also contributes to the elevation of pulmonary artery pressures, although it does not change the pressure gradient across the pulmonary vascular bed (21-27). The aim of the present investigation was to study

1. Central hemodynamics in terms of pressure-flow relationships in patients with obstructive and restrictive lung disease.

2. The factors that contribute to the elevation of the pulmonary artery mean pressure — pulmonary wedge pressure difference, i.e. the pulmonary vascular resistance at rest and during exercise in these patients.

3. An attempt was also made to investigate the general effects of obstructive and restrictive dysfunction on the central hemodynamics.

Procedure

On the day of catheterization the patients had a light breakfast early in the morning and received a sedative two hours before the examination. Right heart catheterization was performed in the recumbent position as described earlier from this laboratory (22, 32). A double lumen catheter was introduced percutaneously according to Jonsson (33) or after exposing an antecubital vein in the left arm. In three cases a single-lumen catheter was used. The catheter was advanced to the pulmonary artery and wedged in a peripheral branch, so that the pulmonary artery and wedge pressures were measured simultaneously. A teflon catheter was introduced percutaneously into the right brachial artery by the Seldinger technique. Measurements of pressures, cardiac output and blood gases were made at rest and during work; the work load for each patient was approximately 2/3 of the working capacity as judged from a previously performed exercise tolerance test. Blood samples were drawn simultaneously from the pulmonary artery and brachial artery for one minute in the middle of the gas collection period for

Table 1 Catheterization data at rest and during exercise in group A. 16 men and 8 women with $\dot{V}O_2$ = oxygen intake, Sa_{O_2} = oxygen saturation of the arterial blood, $\dot{S}\dot{V}O_2$ = oxygen saturation stroke volume RA = right atrium, RV = right ventricle PA = pulmonary artery \bar{P}_w = pulmonary artery pressure, PVR = pulmonary vascular resistance index, SVR = systemic vascular resistance

Subject no.	Sex	Work kpm/min	$\dot{V}O_2$ ml STPD/min	Sa_{O_2} %	$\dot{S}\dot{V}O_2$ %	O_2 capacity ml/l	AVD ml/l	Cardiac output l/min	HR beats/min
1	U.A.	M	rest 50	271 848	94.8 94.2	74.1 36.5	17.70 18.09	37.6 69.2	72.1 12.25
2	K.E.	M	rest 250	258 899	91.7 89.8	68.2 40.9	16.88 17.65	40.7 87.5	6.34 10.30
3	H.F.	M	rest 200	267 824	95.1 96.9	68.9 53.1	18.94 19.91	50.8 89.2	5.26 9.24
4	E.E.	M	rest 100	286 603	94.9 90.1	63.4 51.3	16.78 17.45	53.8 72.2	5.32 8.37
5	W.K.	M	rest 500	324 1064	94.7 91.4	72.7 43.7	17.90 19.99	40.4 96.3	7.3
6	R.K.	M	rest 600	273 1272	97.8 9.6	77.7 43.0	19.12 20.77	40.4 104.0	
7	S.J.	M	rest 400	280 1078	92.8 91.1	72.5 49.5	18.91 19.40	39.4 87.6	
8	W.M.L.	M	rest 150	315 779	86.0 85.8	61.4 44		47.1 82.1	
9	G.F.	M	rest 200	220 428	95.8 95.5	70 42		40.2 83.5	
10	H.M.	M	rest —	240 —	94.2 —	63.5 —		3	
11	E.F.	M	rest 116	272 805	91.5 89.2	61.5 38.7			
12	A.C.	M	rest 116	238 547	49.9 54.4	41.1 27.8			
13	J.I.	M	rest 116	245 547	88.6 81.8	66.2 45.9	20		
14	A.E.	M	rest 116	2.1 544	88.9 66.8	69.0 34.5	19.5 20.76		
15	A.W.	M	rest 1	47 8.6	95.4 96.6	68.8 45.0	17.43 18.38		
16	P.F.	M	rest 130	1 58	91.5 89.4	63.4 39.4	14.87 15.40		

chronic obstructive lung disease.

tion of mixed venous blood, AVD = arteriovenous oxygen difference, HR = heart rate, SV = coronary mean wedge pressure Br₃ = brachial artery S = systolic, D = diastolic, M = mean, distance index.

SV ml	Pressures, mm Hg											SVR
	RA	RV			PA			$\overline{P_w}$	BrA			PVR
		S	Di	M	S	D	M		S	D	M	
87	0.3	24	2	4	10	17	6	131	73	95	2.4	21.0
99		38	4	31	12	24	8	142	78	103	2.1	19.4
85	2	35	2	23	10	16	3	176	76	81	3.8	23.2
96		48	4	45	21	29	7	161	92	118	4.0	20.0
6	4	23	11	20	9	15	9	147	77	100	2.0	32.7
78		53	14	47	24	34	22	184	94	131	2	4.4
62	5	29	7	29	14	20	8	190	103	139	4.2	48.3
77		48	11	46	23	34	14	219	116	165	4.4	36.5
110	5	29	6	28	10	18	11	125	94	84	1.7	70.1
84		62	11	63	25	43	32	156	72	114	2.3	19.8
90	3	22	4	20	9	15	7	127	77	98	3	28.2
87		45	3	33	12	22	6	188	92	129	6	70.6
86	6	27	10	26	11	18	11	110	69	90	1.9	24.6
120		40	12	48	23	33	22	152	82	113	2.1	17.8
86	8	32	10	29	14	21	10	140	90	111	3.4	34.2
98		40	17	39	23	34	21	162	102	134	2.8	29.1
72	10	1	4	24	9	15	6	103	45	76	2.8	23.6
50		43	11	4	19	29	1	164	78	116	3.7	38.4
60	4	5	4	3	10	17	6	114	65	83	4.0	30.4
—		—	—	—	—	—	—	—	—	—	—	—
56	—	76	3	29	11	18	8	149	78	108	4.5	42.9
73		49	15	47	21	32	16	196	90	144	3.8	33.1
117	7	82	8	57	24	38	10	102	61	81	4.2	11.9
73		88	18	88	34	5	16	140	80	102	7.1	19.9
58	2	4	—	26	13	19	5	135	76	97	4.8	34.2
73		54	11	36	30	39	10	163	89	122	5.4	29.7
58	3	36	6	36	19	76	10	133	77	109	4.7	32.2
68		100	17	69	35	50	17	180	103	137	6.3	26.0
63	4	4	4	24	10	16	6	147	96	116	3.3	37.4
79		43	9	41	23	29	10	175	99	130	3.9	25.6
53	6	77	6	29	16	20	11	110	70	92	3.0	34.2
92		49	15	48	23	32	21	119	61	86	1.9	15.1

Subject no		Work Sex kpm/min	$\dot{V}O_2$ ml STPD/ min	S_{aO_2} %	SV_{O_2} %	O_2 capacity ml/l	AVD ml/l	Cardiac output l/min	HR beats/ min
17	S.E.	F rest	224	94.9	69.3	17.67	46.2	4.84	57
		150	673	94.1	46.4	18.93	90.6	7.43	93
18	E.K.	F rest	212	91.9	71.9	16.47	34.0	6.24	77
		100	354	92.6	45.2	16.92	81.2	6.82	96
19	E.A.	F rest	205	98.4	69.7	16.71	49.9	4.11	76
		100	370	89.4	50.1	17.31	69.1	6.23	97
20	B.W.	F rest	233	86.3	65.4	16.73	34.4	6.77	91
		200	639	76.6	39.8	18.25	67.2	9.31	130
21	A.F.	F rest	211	95.4	70.1	15.97	41.3	5.08	63
		150	367	91.3	50.3	17.34	72.1	7.86	92
22	E.P.	F rest	180	94.0	74.3	15.81	31.7	5.68	74
		250	709	91.9	44.6	16.11	77.2	9.18	116
23	A.S.	F rest	169	91.0	67.2	15.89	43.6	3.88	80
		—	—	—	—	—	—	—	—
24	L.H.	F rest	233	91.9	68.9	18.01	42.4	3.31	113
		116	613	89.8	47.2	17.92	77.3	7.93	120

the determination of arterio-venous oxygen difference (AVD). From the brachial artery a blood sample was collected as well immediately after the measurement of the cardiac output, for the determination of P_{aO_2} , P_{aCO_2} and pH. All examinations were carried out without complication except in patient 7 group B who developed a transient complete heart block during exercise in conjunction with measurement of the cardiac output.

Results

Individual observations and data are shown in Tables I–II. Exercise measurements only of 22 of the 1 patient in group A and 16 of the 1 patient in

group B are available, as two patients in the first group and one in the second had to interrupt the exercise shortly after the start because of dyspnea.

In group A the mean work load was 208 kpm/min, range 100–600 and in group B 252 kpm/min, range 116–450.

The oxygen uptake in group A was at rest 240 ml/min, SD \pm 41 and during exercise 731 ml/min, SD \pm 210. In group B the values at rest and during exercise were 258 ml/min, SD \pm 36 and 848 ml/min, SD \pm 234 respectively.

The heart rate in group A averaged at rest 81 beats/min and during exercise 111 beats/min. In group B the average was 81 beats/min and 111 beats/min.

Pressures, mm Hg

SV ml	RA	RV		PA			\bar{P}_w	BrA			PVR	SVR
		S	De	S	ID	M		S	D	M		
83	4	31	7	83	15	21	10	130	73	93	3.6	30.6
80		83	9	50	23	37	11	160	88	120	5.5	25.2
81	3	42	6	32	16	24	7	154	86	113	4.4	29.0
71		49	9	54	23	39	14	185	94	133	5.9	31.2
54	1	24	3	83	15	23	9	158	83	114	5.0	41.0
85		70	11	73	30	45	18	180	83	127	5.9	22.8
72	2	83	6	36	17	26	10	142	81	107	3.6	24.2
72		65	11	60	33	44	24	178	99	135	3.2	21.7
78	2	24	3	23	7	13	4	117	69	93	3.5	29.4
85		44	10	39	17	27	11	138	84	107	3.3	21.9
77	4	23	7	24	8	16	10	112	59	88	1.5	21.7
79		41	11	41	18	29	14	154	79	111	2.3	16.9
49	4	26	6	29	13	18	14	127	69	99	1.5	35.7
—	—	—	—	—	—	—	—	—	—	—	—	—
48	4	29	8	26	10	18	11	121	83	103	2.2	32.9
61		34	8	30	13	21	12	140	82	110	2.0	24.6

In group A the arterial oxygen saturation was 91.2 % at rest, which is slightly lower than normal, and decreased during exercise to 87.9 % $p < 0.05$. The lowest value at rest was 49.9 % found in patient A 12 with secondary polycythemia and severe alveolar hypoventilation with arterial P_{CO_2} of 64 mm Hg. In group B Sa_{O_2} was 94.5 % at rest and decreased during exercise to 90.9 % $p < 0.05$.

The oxygen saturation of the mixed venous blood was in group A 67.7 % at rest and 44.5 % during exercise. In group B it was 70.0 % at rest and 46.3 % during exercise.

The arterio-venous oxygen difference in group A was at rest 43.5 ml/l and

during exercise 84.2 ml/l. In group B it was 43.1 ml/l at rest and 85.2 ml/l during exercise.

The oxygen tension in arterial blood in group A was at rest 64 mm Hg, range 32—93 and during exercise 61 mm Hg, range 35—88 ($p > 0.05$). In group B it was 72 mm Hg, range 63—83 at rest and decreased slightly during exercise ($p < 0.05$) to 66 mm Hg, range 53—83.

The carbon dioxide tension in arterial blood in group A was at rest 43 mm Hg, range 31—69 and during exercise 44 mm Hg, range 32—68. Only 4 patients had elevated $Pa_{CO_2} > 45$ mm Hg at rest and during exercise. The corresponding values in group B were 39 mm Hg, range 34—46 at rest and 40 mm Hg, range 30—47 during exercise.

Table II Catheterization data at rest and during exercise in group B, 12 men and 5 women with

Subject no		Work Set	kpm/min	VO ₂ ml STPD/ min	S _a O ₂ %	S _v O ₂ %	O ₂ capacity ml/l	AVD ml/l	Cardiac output l/min	HR beats/ min
1	H.B.	M	rest 300	280 703	96.8 96.2	69.2 48.0	17.39 17.97	50.0 88.6	5.60 7.96	85 108
2	E.E.	M	rest 200	259 707	98.5 95.6	80.9 64.8	20.69 20.13	38.4 63.0	6.74 12.17	88 110
3	R.U.	M	rest 300	336 1124	93.2 91.2	70.2 47.1	17.26 17.83	40.7 84.7	8.26 13.27	99 135
4	V.S.	M	rest 200	267 838	93.0 72.9	63.3 27.7	17.17 17.58	51.0 79.5	5.21 10.51	81 114
5	J.S.	M	rest 450	277 1214	91.9 91.1	74.2 44.5	18.00 18.97	40.0 89.4	6.93 13.91	75 123
6	B.E.	M	rest 200	268 913	92.5 85.7	63.8 29.8	12.38 24.15	36.5 79.1	7.31 11.57	80 108
7	G.Z.	M	rest 150	270 714	93.7 91.1	67.1 32.5	15.18 16.34	44.4 101.7	6.08 7.07	76 60
8	L.O.	M	rest 200	269 810	93.4 86.8	62.0 40.6	16.59 17.13	53.1 80.2	5.07 10.47	78 114
9	L.P.	M	rest 300	227 1038	91.8 91.8	71.5 46.3	18.41 18.81	43.9 86.6	5.17 11.99	79 95
10	O.M.	M	rest 200	285 791	97.1 96.6	72.3 46.4	18.91 19.70	48.9 100.9	5.83 7.84	78 103
11	I.H.	M	rest —	42 —	93.3 —	63.9 —	17.38 —	52.1 —	1.61 —	91 —
12	A.R.	M	rest 400	307 1196	93.0 91.4	70.5 42.9	18.91 20.06	43.4 101.3	7.01 11.47	70 111
13	C.J.	F	rest 400	235 1103	90.3 83.0	71.7 68.6	17.73 18.73	33.2 —	6.91 —	73 138
14	S.H.	F	rest 100	221 537	90.9 —	63.6 53.0	18.40 19.07	47.6 82.2	4.69 6.80	67 91
15	H.B.	F	rest 116	221 476	92.0 88.6	73.7 56.0	16.15 16.88	30.6 56.1	7.22 8.48	102 122
16	A.D.	F	rest 200	113 46	97.8 96.9	73.1 43.5	16.13 16.87	41.9 92.1	4.61 6.47	86 111
17	I.P.	F	rest 116	113 —	97.8 —	73.4 48.1	17.73 19.10	36.8 89.9	6.06 8.08	96 130

chronic restrictive lung disease. Symbols as in Table I

SV ml	Pressures, mm Hg											SVR
	RA	RV		PA			\bar{P}_w	BA			PVR	
		S	De	S	D	M		S	D	M		
66	1	28	4	31	13	19	7	128	55	81	3.7	24.6
74		48	8	48	23	30	6	183	70	114	5.1	24.4
77	3	26	6	23	7	14	8	117	76	84	1.7	23.7
111		42	6	42	16	29	10	142	87	103	3.0	16.1
83	3	31	7	30	13	20	6	133	82	103	3.0	21.8
98		46	7	46	20	33	13	161	92	124	2.8	16.4
63	3	55	9	54	28	37	15	138	81	100	7.1	32.5
92		85	19	92	48	63	22	153	82	110	6.9	17.7
92	5	33	8	32	12	21	10	115	71	90	3.0	24.1
113		46	12	33	19	33	17	158	84	119	2.4	15.9
92	4	1	7	23	10	16	8	128	87	109	1.9	25.7
107		53	13	58	21	39	20	169	103	136	2.8	20.3
80	3	37	9	33	12	22	8	153	73	100	4.2	29.6
117		68	18	68	32	52	33	203	114	149	4.0	33
63	0	22	1	22	3	13	0	132	79	102	4.9	38.2
92		49	9	40	13	26	4	160	93	132	4.0	24.0
63	9	39	9	36	17	27	12	130	79	102	5.7	39.4
126		66	17	59	23	42	17	179	90	131	4.1	21.8
73	3	42	9	41	16	26	16	161	77	114	3.6	41.0
73		70	19	68	35	51	37	174	91	107	3.8	28.7
49	3	36	8	29	11	19	8	139	69	98	3.9	33.0
—		—	—	—	—	—	—	—	—	—	—	—
101	5	26	8	24	10	18	10	130	78	104	2.2	28.5
102		4	11	40	14	28	13	167	92	119	2.3	20.0
95	6	33	3	29	12	21	11	109	67	87	2.7	23.8
—		50	9	53	20	36	11	136	79	89	—	—
69	10	23	7	23	13	18	9	112	69	91	3.2	32.0
75		32	7	28	15	22	13	110	65	84	2.2	21.2
71	0	29	4	27	12	20	7	137	72	110	3.0	23.3
70		43	6	42	19	31	14	141	79	109	3.1	19.8
54	3	19	4	21	8	13	8	93	58	79	1.7	27.4
57		33	6	34	15	24	13	117	70	92	2.7	22.8
63	3	21	2	20	6	13	6	130	72	93	1.7	27.4
62		50	10	46	23	34	23	194	86	144	2.3	29.9

pH in arterial blood in group A was at rest 7.41 range 7.30—7.48, and during exercise 7.38 range 7.28—7.51 and in group B at rest 7.42, range 7.37—7.49 and during exercise 7.39 range 7.31—7.48. Among the 4 patients in group A with elevated P_{aO_2} , only one (A 12) had a pH lower than 7.35 the other three patients had normal pH.

Intracardiac and intravascular pressures. The individual values are given in Tables I and II mean values \pm SD are given in Table III.

At rest. The mean pressures were compared with mean values reported in healthy elderly subjects aged 61—83 years by Granath *et al.* (22) and Granath and Strandell (23). In group A the systolic and end diastolic pressures in the right ventricle (RV_s , RV_D) pulmonary mean wedge pressure (\bar{P}_W) and the brachial artery systolic (Br_{A_s}) diastolic (Br_{A_d}) and mean ($\bar{P}Br_A$) pressures were not significantly different ($p > 0.05$) from normal values. The pulmonary artery systolic (P_{PA_s}) diastolic (P_{PA_d}) and mean ($\bar{P}PA$) pressure were slightly higher ($p < 0.05$). In group B P_{I_s} and $\bar{P}PA$ were slightly higher than normal ($p < 0.05$) while other pressures were not significantly different ($p > 0.05$).

During exercise. As the work load in this material differed from that for the healthy elderly subjects mentioned above, the regression lines of the pressures on the cardiac output (including the values both at rest and during exercise) were compared with the two materials (Figs. 1–5). In both groups the

regression line of RV had a slope that was not significantly different from that in normal subjects ($p > 0.05$). The intercept was lower in group A ($p < 0.01$) in group B higher ($p < 0.05$). The regression lines of RV_D in both groups were identical with that in normal subjects (Fig. 1).

The $\bar{P}PA$ regression line had approximately the same slope as in healthy subjects ($p > 0.05$) but the intercept was significantly higher in group A ($p < 0.001$) and in group B ($p < 0.01$).

Fig. 2. In both groups \bar{P}_W values during exercise were within normal limits except in two patients in group B (Nos. 7 and 10) and in one patient in group A (No. 5) for whom it was higher than $+2$ SD. These patients had signs of myocardial disease. Two other patients, one from each group, had a lower P_W than -2 SD. The slope of \bar{P}_W on the cardiac output in group B was slightly lower than in normal subjects ($p < 0.05$) while the intercept was not significantly different (Fig. 3). In group A no significant difference was found in slope or intercept compared with normal subjects. The regression of Br_A on the cardiac output was in both groups not significantly different from the regression in normal subjects (Fig. 4 and 5). However, most of the pressures in both groups showed a wide individual variation from normal to highly elevated.

As $\bar{P}PA$ was elevated in the two groups both at rest and exercise, while \bar{P}_W was normal, the difference between the two pressures ($\bar{P}PA - \bar{P}_W$) was significantly larger in the patient material than in nor-

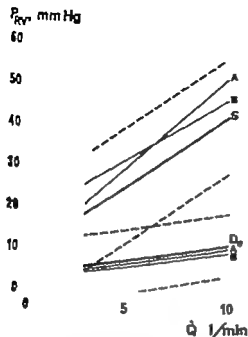


Fig 1 Regression of right ventricular systolic (S) and end diastolic (De) pressures on cardiac output (\dot{Q}) at rest and during exercise in group A and in group B. Thick solid lines and interrupted lines are regression lines ± 2 SD for healthy elderly subjects (22, 23)

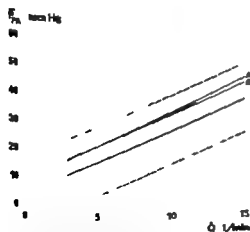


Fig 2 Regression of pulmonary artery mean pressure (P_{PA}) on cardiac output at rest and during exercise. Material and symbols as in Fig 1

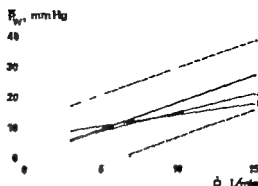


Fig 3 Regression of mean wedge pressure (P_W) on cardiac output at rest and during exercise. Material and symbols as in Fig 1

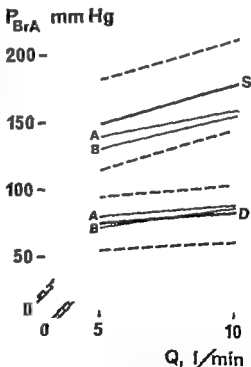


Fig 4 Regression of brachial artery systolic (S) and diastolic (D) pressures on cardiac output at rest and during exercise. Material and symbols as in Fig 1

\bar{P}_{BrA} mmHg
140

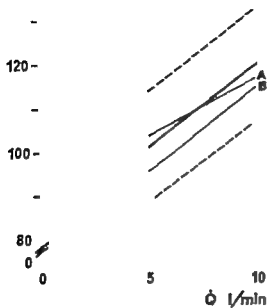


Fig 5 Regression of brachial artery mean pressure (\bar{P}_{BrA}) on cardiac output : rest and during exercise. Material and symbols as in Fig 1

$\bar{P}_{PA} - \bar{P}_W$, mmHg

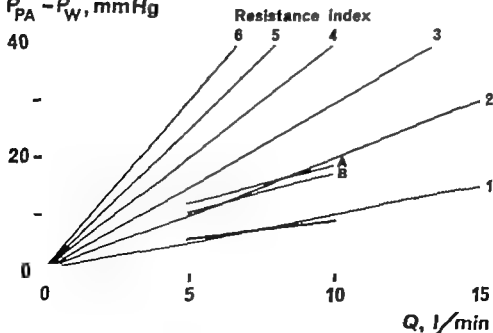


Fig 6 Regression of the pressure difference ($\bar{P}_{PA} - \bar{P}_W$) on cardiac output at rest and during exercise. Material and symbols as in Fig. 1

mal material (Fig 6) $\bar{P}_{PA} - \bar{P}_W$ was identical in the two groups and averaged at rest 11 mm Hg, increasing significantly during exercise to an average of 19 mm Hg. The mean vascular pressures in groups A and B are given in Table III. There was no significant difference between the group means as regards these pressures either at rest or during exercise. Also there was no significant difference between the slopes of pressures on the cardiac output at rest and exercise in the two groups.

The pulmonary vascular resistance in *dex* was, in group A, 3.3 units at rest and increased slightly during exercise to 3.9 units ($p < 0.05$). In group B the average was 3.4 units at rest and 3.5 units during exercise ($p > 0.05$). The values were significantly higher than values reported in healthy elderly subjects (23).

Parameters for the pressure difference ($\bar{P}_{PA} - \bar{P}_W$)

$\bar{P}_{PA} - \bar{P}_W$ at rest in group A was significantly correlated with Sa_{O_2} ($r = -0.803$), Pa_{O_2} ($r = -0.731$) and Pa_{CO_2} ($r = 0.782$) in linear regression analysis (for all values $p < 0.001$). A significant correlation was also found during exercise with Sa_{O_2} ($r = -0.681$), Pa_{O_2} ($r = -0.660$) ($p < 0.001$) and Pa_{CO_2} ($r = 0.624$) ($p < 0.01$). No significant correlation was found with pH either at rest or during exercise. In group B a significant correlation was found between $\bar{P}_{PA} - \bar{P}_W$ and Sa_{O_2} during exercise only ($r = -0.807$) ($p < 0.01$) and with Pa_{O_2} ($r = -0.638$) ($p < 0.05$). At

rest and during exercise no significant correlation was found with Pa_{CO_2} and pH (Table IV).

In group A the pulmonary artery diastolic pressure — pulmonary mean wedge pressure difference ($P_{PA_d} - \bar{P}_W$) was significantly correlated with Sa_{O_2} and Pa_{CO_2} both at rest and during exercise (Table IV). In group B there was a significant correlation during exercise only. The correlation coefficients for blood gases with $P_{PA_d} - \bar{P}_W$ were lower than those with $\bar{P}_{PA} - \bar{P}_W$. In neither group was a correlation found with pH nor was the pulmonary mean wedge pressure correlated with Sa_{O_2} , Pa_{CO_2} or pH.

In group A the cardiac output in men at rest was 6.18 l/min, SD ± 1.64 , $n = 16$ and during exercise 9.34 l/min, SD ± 2.00 , $n = 15$. The corresponding cardiac indexes were $3.50 \text{ l/m}^2 \pm 1.03$ and $5.25 \text{ l/m}^2 \pm 1.11$. In women the figure at rest was 5.27 l/min, SD ± 0.99 , $n = 8$ and during exercise 8.14 l/min, SD ± 0.94 , $n = 7$ and the corresponding cardiac indexes were $3.42 \text{ l/m}^2 \pm 0.62$ and $5.25 \text{ l/m}^2 \pm 0.89$. The relationship between cardiac output and oxygen uptake at rest and during exercise (Fig 7) was within ± 2 SD for the regression line in healthy elderly subjects (22) except in one patient (A 12) in whom it was higher than $+2$ SD. He had severe hypoxemia. The relationship between cardiac output and oxygen uptake for all observations both at rest and during exercise followed the equation $Q = 4.20 + 6.6 V_{O_2}$, $r = 0.851$, SD ± 1.20 , $n = 46$, $p < 0.001$. The regression of the

T 4/ III Mean pressures \pm SD in group A and in group B at rest (R) and during exercise (E)

For symbols, see text.

	R\	PA			\bar{P}_w			BrA		$\bar{P}_{A_2} - \bar{P}_w$	
		S	D ₀	S	M	D	M	D	M		
A	R	-24	29.8 \pm 12.3	5.5 \pm 2.3	28.5 \pm 7.5	12.6 \pm 4.0	19.5 \pm 5.2	8.5 \pm 7	131.3 \pm 20.0	74.8 \pm 12.5	99.0 \pm 14.1
	E	-22	52.1 \pm 13.5	10.7 \pm 4.5	50.0 \pm 14.0	23.0 \pm 6.5	34.6 \pm 8.6	15.4 \pm 6.4	165.5 \pm 22.8	88.0 \pm 11.9	121.9 \pm 16.7
B	R	-17	30.4 \pm 9.5	6.2 \pm 2.7	29.5 \pm 8.6	12.1 \pm 5.2	19.8 \pm 6.1	8.8 \pm 3.6	128.5 \pm 16.2	73.2 \pm 8.2	97.2 \pm 10.3
	E	-16	51.6 \pm 14.0	11.3 \pm 4.6	51.1 \pm 15.6	22.5 \pm 9.1	36.1 \pm 11.5	16.6 \pm 8.8	159.9 \pm 26.0	86.2 \pm 12.8	116.4 \pm 19.2

T 4/ I Relationships between pressures and blood gases in group A and in group B at rest (R) and during exercise (E) For symbols, see text.

	R\	\bar{P}_{A_2}			P_{A_2}			$\bar{P}_{A_2} \bar{P}_w$			$P_{A_2} \bar{P}_w$	
		R	E	S	R	E	M	R	E	M	R	E
Group A	SeO ₂	-0.818	-0.716	-0.692	-0.692	-0.691	-0.681	-0.803	-0.681	-0.593	-0.565	-0.565
	P	+++	++	+++	+++	+++	+++	+++	+++	++	++	++
Group A	P _{ACO₂}	21	22	24	24	22	24	24	22	24	24	22
	P	0.808	0.704	0.709	0.709	0.718	0.694	0.782	0.694	0.600	0.446	0.446
		+++	++	++	++	+++	++	++	++	++	+	+
Group B	SeO ₂	-0.175	-0.506	-0.202	-0.202	-0.461	-0.292	-0.292	-0.807	-0.319	-0.645	-0.645
	P	---	---	---	---	---	---	---	---	---	---	---
Group B	P _{ACO₂}	17	15	17	17	15	17	17	15	17	17	15
	P	0.251	-0.155	0.256	0.256	-0.155	0.407	0.407	0.513	0.427	0.659	0.659
	n	---	---	---	---	---	---	---	---	---	---	---
		14	13	14	14	13	14	14	13	14	14	13

cardiac output on oxygen uptake had approximately the same slope as in healthy young subjects (13) but the intercept was obviously lower (Fig 9). However neither slope nor intercept differed when compared to the regression line found in healthy elderly subjects (22).

In group B the cardiac output in men at rest was 6.16 l/min , $\text{SD} \pm 1.10$, $n = 12$, and during exercise 10.74 l/min , $\text{SD} \pm 2.27$, $n = 11$. The corresponding cardiac index was $3.37 \text{ l/m}^2 \pm 0.65$ and $5.83 \text{ l/m}^2 \pm 1.29$. In women the cardiac output was at rest 5.88 l/min , $\text{SD} \pm 1.24$, $n = 5$ and during exercise 7.46 l/min , $\text{SD} \pm 0.97$, $n = 4$; the corresponding cardiac index being $3.54 \text{ l/m}^2 \pm 0.75$ and $6.64 \text{ l/m}^2 \pm 0.67$. In one female patient the cardiac output during exercise was discarded. At rest and during exercise the relationship between the cardiac output and oxygen uptake was within $\pm 2 \text{ SD}$ except in patient B 2 whose cardiac output during exercise was $>2 \text{ SD}$ (Fig 8). The regression of cardiac output on the oxygen uptake at rest and during exercise was according to the following equation: $Q = 4.12 + 7.1 V_{O_2}$, $r = 0.886$, $\text{SD} \pm 1.26$, $n = 32$, $p < 0.001$. The regression line had a slope of 7.1 compared to 6.6 in group A, a difference which was not significant ($p > 0.05$). Also the intercept was approximately the same in both groups (Fig 9).

In group A the stroke volume in men at rest was 76.1 ml , $\text{SD} \pm 20.2$, $n = 16$ and during exercise 82.8 ml , $\text{SD} \pm 16.5$, $n = 15$; in women 68.0 ml , $\text{SD} \pm 15.1$, $n = 11$ at rest and 76.1 ml , $\text{SD} \pm$

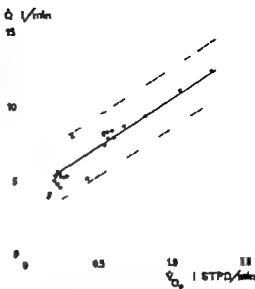


Fig 7 Cardiac output in relation to oxygen uptake at rest ($n = 24$, open symbols) and during exercise ($n = 22$, solid symbols) in patients with obstructive lung disease. Regression line $\pm 2 \text{ SD}$ for healthy elderly subjects (22, 23)

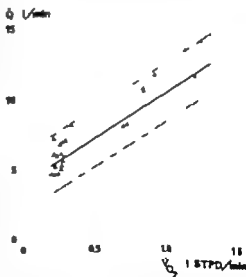


Fig 8 Cardiac output in relation to oxygen uptake at rest ($n = 17$, open symbols) and during exercise ($n = 15$, solid symbols) in patients with restrictive lung disease. Regression line $\pm 2 \text{ SD}$ as in Fig. 7

87 $n = 7$ during exercise. There was no significant difference between females and males either at rest or during exercise ($p > 0.05$). The average increase in stroke volume when only paired observations were included was 8 % in men and 7 % in women. The mean values at rest and during exercise were significantly lower than values reported in healthy young subjects by Bevegård *et al* (4) and Holmgren *et al* (32). The stroke volume in men at rest did not differ significantly from values reported in healthy elderly subjects by Granath *et al* (22) but during exercise it was significantly lower. The stroke volume in relation to THb and HV during supine exercise was in most of the patients lower than -2 SD of the regression line found in healthy young subjects (4 5 31 32). The relationship between the stroke volume and THb is shown in Fig. 10. The stroke volume during exercise was positively correlated with THb and PVR ($p < 0.05$) but not significantly with BV, HV, \bar{P}_{PA} , $\bar{P}_{PA} - \bar{P}_{PW}$ or SA_{O_2} .

In group B the stroke volume in men was at rest 78.3 ml, SD ± 17.7 , $n = 11$ and during exercise 100.6 ml, SD ± 16.5 , $n = 11$. In women the values were 64.3 ml, SD ± 7.6 , $n = 4$ at rest and 66 ml, SD ± 8 , $n = 4$ during exercise. The increase in the stroke volume between rest and exercise averaged in men 28 % and in women 3 %. The relationship between the stroke volume during exercise and THb and HV was within ± 2 SD except in two males and three females, who had small stroke volumes in relation to their THb and HV. The relationship between the stroke volume and THb is shown in Fig. 11. One of

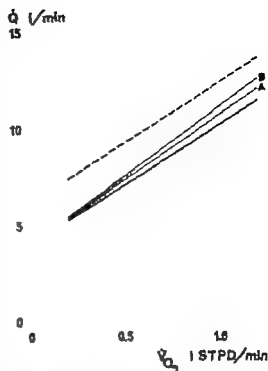


Fig. 9 Regression lines for cardiac output on oxygen uptake at rest and during exercise in group A and in group B compared with regression lines for healthy elderly (solid line) and young (interrupted line) subjects.

these male patients (B 4) had left bundle branch block and the other (B 10) had ST depression in his exercise ECG. The three females had normal heart volumes in relation to total amount of hemoglobin. In the fifth female patient (B 13) the stroke volume, measured only at rest, was 95 ml, which was normal in relation to THb and heart volume. In group B the stroke volume during exercise was significantly correlated with THb ($p < 0.05$) but no correlation was found with BV, HV, \bar{P}_{PA} , $\bar{P}_{PA} - \bar{P}_{PW}$, PVR, SA_{O_2} or VC % of predicted. In men the stroke volume during exercise was significantly larger

Stroke volume ml
during supine exercise

150

100

50



Fig 10 Stroke volume during supine exercise in relation to total amount of hemoglobin of women (open symbols) and 15 men (solid symbols) with obstructive lung disease. Regression line ± 2 SD for healthy young subjects (4 5 31 32)

Stroke volume ml
during supine exercise

150

100

50



Fig 11 Stroke volume during supine exercise in relation to total amount of hemoglobin of 4 women (open symbols) and 11 men (solid symbols) with restrictive lung disease. Regression line ± 2 SD as in Fig. 10.

in the restrictive than in the obstructive group ($p < 0.02$) but no significant difference was found at rest ($p > 0.05$). In women there were no significant differences between the two groups either at rest or during exercise. This might be due to the small number of female patients in the restrictive group.

In order to demonstrate the effect of the degree of airway obstruction on the hemodynamics, group A was divided into 3 subgroups according to the FEV₁ % (of predicted). Table V. Subgroup I included patients with FEV₁ % more than 75 % mean 82 % $n = 4$ subgroup II with FEV₁ % 51–75 % mean

65% $n = 10$ and subgroup III with FEV₁ % ≤ 50 % mean 47 % $n = 10$. Patient 14 A.E., was included in subgroup III as he had a very low VV_{max} (15 l/min) although FEV₁ % was 61 % of the predicted value.

At rest and during exercise with increasing grade of airway obstruction (decreasing FEV₁ %) there was a decrease in SA_{O_2} without significant difference between the subgroup means. There was a correlation of probable significance between FEV₁ and SA_{O_2} ($p < 0.05$) for the whole group during exercise, but no significant correlation was found at rest. Also FEV₁ was linearly correlated with

TABLE V The relative effects of obstructive ventilatory dysfunction upon central hemodynamics.

		SaO_2		PaCO_2		\bar{P}_w	
		R	L	R	L	R	L
I	FEV % > 75 % predict.	93.2±1.8 (4)	91.3±1.7 (4)	40.0±2.9 (4)	39.8±4.6 (4)	10.0±2.2 (4)	18.5±11.0 (4)
II	FEV % 51—75 % predict.	94.8±1.5 (10)	93.6±2.4 (9)	38.8±5.7 (10)	40.4±5.8 (9)	8.3±2.9 (10)	14.2±5.2 (9)
III	FEV % ≤ 50 % predict.	86.1±13.0 (10)	80.7±13.0 (9)	47.3±10.3 (10)	49.1±12.1 (9)	8.0±2.7 (10)	15.2±5.3 (9)

TABLE VI The relative effects of restrictive ventilatory dysfunction upon central hemodynamics.

		SaO_2		PaCO_2		\bar{P}_w	
		R	L	R	L	R	L
(a)	VC 63 % pred range 51—72	91.2±2.2 (9)	91.1±8.1 (8)	37.0±3.3 (7)	37.4±5.3 (7)	9.4±3.7 (9)	20.0±10.1 (9)
(b)	VC 41 % pred range 29—47	91.8±2.8 (8)	90.5±12.2 (7)	40.4±4.1 (7)	42.6±4.8 (7)	8.0±3.7 (8)	12.3±4.3 (7)

PaO_2 both at rest and during exercise ($p < 0.05$). Subgroup III had slightly higher PaCO_2 than subgroup II at rest ($p < 0.05$) but not during exercise ($p > 0.05$). There was no significant linear correlation between FEV_1 and PaCO_2 for the whole group either at rest or during exercise.

The pulmonary artery mean pressure (\bar{P}_{PA}) was 5 mm Hg higher in subgroup III than in subgroup I at rest and 6 mm Hg during exercise but there was hardly any difference between subgroups I and II. The pulmonary mean wedge pressure (\bar{P}_w) did not show any significant difference between the subgroups either

at rest or during exercise. Thus the pressure difference ($\bar{P}_{PA} - \bar{P}_w$) increased with decreasing FEV % (Fig. 12). At rest a significant difference in $\bar{P}_{PA} - \bar{P}_w$ was found between subgroups I and III and between subgroups II and III ($p < 0.05$). During exercise there was an increase from 13.8 mm Hg in subgroup I to 22.6 mm Hg in subgroup III. FEV_1 was negatively correlated with $\bar{P}_{PA} - \bar{P}_w$ both at rest and during exercise ($p < 0.05$). It followed that the pulmonary vascular resistance (PVR) at rest showed a significant difference between subgroups I and III ($p < 0.01$) and between subgroups II and III ($p < 0.05$).

Mean values \pm SD number of observations in parentheses.

$\overline{P_{PA}}$		$\overline{P_{PA}} - \overline{P_w}$		C.I.		PVR	
R	E	R	E	R	E	R	E
17.3 \pm 2.2	32.0 \pm 9.6	7.3 \pm 1.0	13.8 \pm 4.4	3.60 \pm 0.68	6.07 \pm 0.42	2.1 \pm 0.7	3.3 \pm 0.3
(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
17.6 \pm 3.1	32.7 \pm 6.3	9.3 \pm 3.0	18.4 \pm 5.2	3.22 \pm 0.52	5.24 \pm 1.29	2.9 \pm 1.1	3.8 \pm 1.4
(10)	(9)	(10)	(9)	(10)	(9)	(10)	(9)
22.4 \pm 6.6	37.8 \pm 10.0	14.4 \pm 5.7	22.6 \pm 9.1	3.70 \pm 1.23	4.90 \pm 0.74	3.8 \pm 0.7	4.7 \pm 2.0
(10)	(9)	(10)	(9)	(10)	(9)	(10)	(9)

Mean values \pm SD number of observations in parentheses.

$\overline{P_{PA}}$		$\overline{P_{PA}} - \overline{P_w}$		C.I.		PVR	
R	E	R	E	R	E	R	E
20.9 \pm 7.2	39.3 \pm 15.9	11.4 \pm 4.8	19.3 \pm 10.0	3.58 \pm 0.62	5.90 \pm 1.35	3.4 \pm 1.6	3.6 \pm 1.6
(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)
18.5 \pm 4.9	31.9 \pm 6.3	10.3 \pm 3.5	19.6 \pm 5.0	3.32 \pm 0.72	5.83 \pm 1.14	3.3 \pm 1.4	3.2 \pm 0.7
(8)	(7)	(8)	(7)	(8)	(6)	(8)	(6)

During exercise the difference was mainly between subgroups I and III ($p < 0.01$). The correlation between FEV₁ and PVR for the whole group was significant at rest ($r = -0.546$ $p < 0.01$) and during exercise ($r = -0.506$ $p < 0.05$). The cardiac output was not influenced by airway obstruction either at rest or during exercise. The stroke volume averaged in subgroups I—III at rest 48, 43 and 42 ml/m² and the corresponding values during exercise were 55, 49 and 44 ml/m². There was no significant difference between the subgroup means ($p > 0.05$). There was no significant correlation between FEV₁ and the stroke volume either at rest or

during exercise ($p > 0.05$).

To study the effect of restrictive ventilatory dysfunction on the hemodynamics, group II was divided into two subgroups (a) VC > 50 % of predicted, mean 63 \pm 9, mean age 58 years, and (b) VC < 50 % of predicted mean 41 \pm 8, mean age 50 years (Table VI). SaO₂ did not show a significant difference between the subgroups. There was no significant correlation between SaO₂ or PaO₂ and VC either at rest or during exercise. But there was a slightly significant negative correlation between PaCO₂ and VC both at rest and during exercise ($p < 0.05$). PaCO₂ was slightly higher in (b) than in (a). At rest $\overline{P_w}$

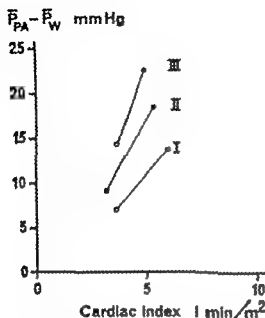


Fig. 12 Mean pressure difference $\bar{P}_{PA} - \bar{P}_W$ in relation to cardiac index at rest and during exercise in patients with obstructive lung disease

- I patients with FEV₁ % (of predicted) > 75
- II patients with FEV₁ % (of predicted) 51-75
- III patients with FEV₁ % (of predicted) ≤ 50

and \bar{P}_{PA} did not show a significant difference between subgroup (a) and subgroup (b). During exercise \bar{P}_W measured 20 mm Hg in subgroup (a) and 12.3 mm Hg in subgroup (b) and \bar{P}_{PA} measured 39.3 mm Hg in (a) and 31.9 mm Hg in (b). The difference was not significant ($p > 0.05$). The $\bar{P}_{PA} - \bar{P}_W$ during exercise was virtually identical in (b) and (a). The cardiac output and the stroke volume were not affected by the decrease in vital capacity.

Discussion

Chronic lung disease causes changes in the pulmonary circulation through structural or functional changes of the pulmonary vessels, leading to an increased vascular resistance and elevation of pulmonary artery pressure. An elevation in \bar{P}_W may contribute to the increase of pulmonary artery pressure (21, 27, 38). In both groups \bar{P}_W normal at rest increased during exercise, contributing to about 50 % of the rise in the pulmonary artery mean pressure. It is preferable therefore to study pressure changes, particularly during exercise, on the basis of changes in the pressure gradient across the pulmonary vascular bed (21, 27, 35) as elevation of \bar{P}_W does not contribute to the pressure gradient (27). This study demonstrated a slightly elevated pressure difference $\bar{P}_{PA} - \bar{P}_W$ at rest in both groups, which increased 8 mm Hg during exercise. It also demonstrated in the obstructive group a slight oxygen desaturation at rest, which increased slightly during exercise. In the restrictive group the oxygen saturation at rest was normal and decreased slightly during exercise.

Following the demonstration of pressure increase on induced hypoxia in cat (16) and in man (19, 40) many investigators confirmed the importance of hypoxemia as a factor in the genesis of pulmonary hypertension in chronic lung disease (1, 15, 24, 26, 43). The results of this study add further confirmation to the previous findings. The absence of a significant correlation at rest in the restrictive group was probably due to the normal oxygen saturation at rest in these

patients. In 23 patients of 43 with pulmonary fibrosis reported in the literature and reviewed by Wade and Bishop (44) the Sa_{O_2} was normal at rest. Also in patients with pleural restriction secondary to pulmonary tuberculosis the oxygen saturation at rest was found to be normal (39).

An increase in arterial P_{CO_2} has been implicated as a cause of pulmonary hypertension in patients with chronic lung disease when breathing carbon dioxide mixtures (20-42) or by hypoventilation when breathing oxygen in normal subjects (34). In the obstructive group there was a significant correlation between $\bar{\text{P}}_{\text{PA}} - \bar{\text{P}}_{\text{W}}$ and Pa_{CO_2} at rest and during exercise. The correlation coefficient was approximately of the same order as between these parameters and Sa_{O_2} (Table IV). Emungil *et al.* (14) in a similar study showed that the correlation between Pa_{CO_2} and both the pulmonary pressure and the pulmonary vascular resistance was always lower than that with Sa_{O_2} . The absence of a significant correlation in the restrictive group is due to the absence of hypercapnia in these patients.

In the obstructive group Sa_{O_2} and Pa_{CO_2} were correlated at rest ($r = -0.580$ $p < 0.01$) and during exercise ($r = -0.658$ $p < 0.001$). This, together with the significant correlation between these variables and $\bar{\text{P}}_{\text{PA}} - \bar{\text{P}}_{\text{W}}$ shows that the elevated $\bar{\text{P}}_{\text{PA}} - \bar{\text{P}}_{\text{W}}$ is mainly a consequence of deranged gas exchange inherent in these patients.

Several investigators have demonstrated the significance of an increase in hydrogen ion concentration in the genesis

of pulmonary hypertension (15, 28, 36). In this study no significant correlation was found between $\bar{\text{P}}_{\text{PA}} - \bar{\text{P}}_{\text{W}}$ or the pulmonary artery mean pressure and pH in either group. This might be due to the fact that the change in pH was small in this material. The lack of significant correlation however is in accordance with other studies, showing that patients with chronic obstructive lung disease usually maintain a fairly normal pH (2, 14). For this reason some investigators did not find a significant correlation between the pulmonary artery pressure and pH in chronic obstructive disease (2). In 8 patients with chronic bronchitis with acute respiratory failure Abraham *et al.* (2) found no significant correlation between pH and pulmonary artery pressure either during the acute illness or recovery.

It has been postulated that the reduction in the pulmonary vascular bed plays a minor role in the development of pulmonary hypertension in chronic obstructive lung disease (18, 29). This was based on the following findings. 1) Removal of 50% of the vascular bed results in only slight elevation of resting pulmonary artery pressure (10). 2) There was no correlation between the degree of anatomic destruction and right ventricular hypertrophy in patients with emphysema (11). 3) The pulmonary arterial hypertension is reversible (29). However the elevation of $\bar{\text{P}}_{\text{PA}} - \bar{\text{P}}_{\text{W}}$ in the restrictive group at rest to the same level as in the obstructive group in the present study in spite of normal blood gases, points to the importance of a restricted vascular bed as a cause of pulmonary hypertension. Simi-

larly the elevation of the pressure gradient during exercise was too high to be caused by the slight hypoxemia present in this group.

Harvey *et al* (27-30) studied the pressure flow relationship in terms of diastolic pressure gradient and found an increase in the diastolic pressure gradient with decreased oxygen saturation and increased hydrogen ion concentration. In the present study the correlations between Sa_{O_2} or Pa_{CO_2} and $\bar{P}_{\text{PA}} - \bar{P}_{\text{w}}$ were higher than those between the blood gases and $\text{P}_{\text{PA}_d} - \bar{P}_{\text{w}}$ (Table IV). This might be attributed to the fact that the pulmonary artery mean pressure is more sensitive to changes in blood gases than is the pulmonary artery diastolic pressure and to technical difficulties in measuring the diastolic pressure, particularly in the presence of the ventilatory swings of pressures.

There has been a controversy as to whether the cardiac output in chronic obstructive lung disease is normal, high or low (6, 17, 26, 44). It has been demonstrated that cardiac output decreases with age in normal subjects (7, 22) and this was suggested as a factor responsible for the disagreement regarding cardiac output in obstructive lung disease (37). This is illustrated in the present study in which the regression lines of the cardiac output on the oxygen uptake were compared with the regression lines in healthy young (13) and elderly subjects (22). Fig. 9.

The stroke volume was virtually normal at rest in both groups. When both males and females were included, the increase in stroke volume from rest to

exercise was 8 % in the obstructive group ($p > 0.05$) which is about 50 % lower than the increase reported in elderly male subjects (22). The increase in the restrictive group was 23 % ($p < 0.01$).

The stroke volume depends on the filling and emptying conditions of the heart. The pressure load to which the right and left hearts were subjected was essentially identical in the two groups. Furthermore the right and left ventricular filling pressures were identical in both groups. Thus the difference in stroke volume is probably to be attributed to the increase in intrathoracic pressure which alters the negative pressure surrounding the heart to zero (atmospheric) or positive pressure (9, 38) thereby reducing the effective filling pressure of the central capacity vessels and of the cardiac chambers. Nakhjavan *et al* (41) have demonstrated that, in patients with emphysema with hyperinflation of the lungs and a low diaphragmatic position the blood flow into the thorax was greatly reduced or completely arrested during inspiration. Another contributing factor is that physical inactivity results in small blood volume and instability of the tone of capacity vessels. One or more of these factors may explain the smaller stroke volume during exercise in patients with obstructive disease.

As previously mentioned, the pulmonary artery wedge pressure was in both groups within the normal range compared to values reported in elderly subjects, although it is high compared to healthy young

(22) ascribed the high wedge pressure in healthy elderly subjects to the lower distensibility of the myocardium as the stroke volume increased normally during exercise. The low compliance of the myocardium may also explain the high wedge pressure during exercise in the restrictive group which also had a normal increase of stroke volume. In the obstructive group, however the high wedge pressure during exercise was associated with a small stroke volume. This might reflect a left ventricular dysfunction. However in these patients the ECG during exercise did not show any significant signs of myocardial disease although this does not exclude its presence. Some investigators have shown that the left ventricular function was not impaired in chronic lung disease (12, 45). Recently however Baum *et al* (3) demonstrated a left ventricular dysfunction in most of the patients with chronic obstructive lung disease studied by them.

There are few published reports on the effects of obstructive or restrictive ventilatory dysfunction on the central hemodynamics. In the obstructive group a close relationship was recorded between the degree of airway obstruction and the pressure difference across the pulmonary vascular bed ($\bar{P}_F - \bar{P}_w$) but in spite of this no significant effect was found on the cardiac output. Consequently the pulmonary vascular resistance increased with increasing airway obstruction. There was also a close relationship between airway obstruction and the degree of hypoxemia. This is in agreement with other studies (14). Harris *et al* (25) demonstrated an increase in pulmonary vascular resistance by

hyperventilation in patients with chronic bronchitis and attributed the increase in pressure gradient and pulmonary vascular resistance to the mechanical effect of hyperventilation in the presence of increased airway resistance. In contrast to their results the blood gases in this study were abnormal while ventilation was only slightly increased. It emerges that the large $\bar{P}_F - \bar{P}_w$ and consequent PVR, could be ascribed either to the effect of blood gases or to the increase in intrathoracic pressure, or most probably to both factors.

Linn and Brownlee (37) and Lockhart *et al* (38) found some evidence that \bar{P}_w increases with increasing intrathoracic pressure in chronic obstructive lung disease. However in contrast to their findings and those of Harris *et al* (25) \bar{P}_w in the present study did not increase with decreasing FEV₁ and the increase in $\bar{P}_F - \bar{P}_w$ was essentially caused by an increase in \bar{P}_F . There is a probability that the effect of increased intrathoracic pressure on the wedge pressure in this study was concealed by the counter-effect of the decreased central blood volume.

At rest there was no effect on central hemodynamics with increasing degree of restriction. During exercise it was shown that, at approximately identical levels of cardiac output, the mean pulmonary artery and wedge pressures were lower in patients with marked degree of restriction (b) than in patients with less degree of restriction (a). The difference in age between the two subgroups was too small to account for the difference in pressures. Also both subgroups had

the same degree of hypoxemia, while Pa_{CO} was higher in (b) so that the blood gases cannot be responsible for it. It seems likely that the low pressures in patients with marked restriction are probably caused by the effect of high negative intrapleural pressure particularly during inspiration, on the transmural pressure (8). As the effect on the pulmonary artery pressure and wedge pressure during exercise was of the same magnitude, the pressure difference $\bar{P}_{\text{PA}} - \bar{P}_{\text{W}}$ and pulmonary vascular resistance were virtually unchanged. This finding might explain the observation made by Wade and Bishop (44) that in pulmonary fibrosis the hypoxemia during exercise is accompanied by a relatively mild pulmonary hypertension.

Summary

- 1 The cardiac output was normal in relation to oxygen uptake at rest and during exercise in both the obstructive (A) and restrictive (B) groups. It was high in one patient in A and during exercise only in one patient in B.
- 2 The stroke volume increased from rest to exercise by 8% ($p > 0.05$) in group A and by 23% ($p < 0.01$) in group B.
- 3 In both groups the mean intracardiac and intravascular pressures at rest were normal except for a slightly elevated \bar{P}_{PA} . During exercise most pressures (apart from wedge pressure) increased from normal to markedly elevated. The \bar{P}_{PA} and PAH also increased from normal to

mm Hg at rest and 19 mm Hg during exercise in both groups.

- 4 $\bar{P}_{\text{PA}} - \bar{P}_{\text{W}}$ was highly significantly correlated with the degree of arterial desaturation and Pa_{CO} increase in group A but respectively less and non-significantly in group B.
- 5 $\bar{P}_{\text{PA}} - \bar{P}_{\text{W}}$ and PVR increased with increasing degree of obstruction, but were virtually unchanged with increasing degree of restriction.
- 6 In neither group was the cardiac output affected by the ventilatory dysfunction.

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CHAPTER VI

DETERMINANTS OF PHYSICAL WORKING CAPACITY IN OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

The study here reported is based on the results presented in Chapters III—V. It was demonstrated in patients with obstructive disease that the physical working capacity (W_B) was closely associated with low ventilatory capacity and in restrictive patients was related to the blood volume. It was also demonstrated that the diffusing capacity of the lung was reduced and the pulmonary vascular resistance increased during exercise in the two groups. The purpose of the present study was to determine the relative importance of the measures of lung function, diffusing capacity and some cardiovascular factors, such as the pulmonary vascular resistance and stroke volume for the determination of physical working capacity in patients with obstructive and restrictive lung disease.

Results

The data have been reported in the respective chapters, and mainly the results of the correlation study will be mentioned.

Matrix correlation and multiple correlation analysis were performed using 10 variables in the obstructive group (A) and 13 variables in the restrictive

group (B) (Tables I—II). In group A the highest correlation was found between W_B and the indices of ventilatory capacity and D_{LCO} ($p < 0.001$). W_B was also significantly correlated with PVR and PaO_2 ($p < 0.01$). The correlations between W_B and SV, SaO_2 were only of probable significance ($p < 0.05$).

Using two independent variables together with W_B as a dependent variable in a multiple regression analysis there was no improvement in the correlation when FVC was combined with either FEV or MVV₆₀. The inclusion of D_{LCO} with FEV in the regression analysis increased the correlation coefficient from 0.819 to 0.914. When D_{LCO} was combined with MVV₆₀, the correlation coefficient increased from 0.858 to 0.942 and the standard error of estimate decreased from 106 to 71.

The regression equations for the above relationships are written

$$W_B = 14.56 + 5.2 \text{ MVV}_{60} + 13.11$$

$$D_{LCO} \text{ SE } 71 \quad r = 0.942$$

$$W_B = 27.67 + 154.63 \text{ FEV}_1 + 13.98 D_{LCO} \text{ SE } 86 \quad r = 0.914$$

When SV was combined with either FEV₁ or MVV there was no improve

TABLE I Correlation matrix in group A

	W_1	IRC	FLV	ANVA	D_{100}	P_{100}	PVR	$\bar{P}_{TA} - \bar{P}_W$	SV	SaO_2
W_1	1.000 (21)									
IRC	0.850 (1)	1.000 (24)								
FLV	0.823 (21)	0.878 (21)	1.000 (21)							
ANVA	0.818 (24)	0.914 (24)	0.898 (24)	1.000 (4)						
D_{100}	0.010 (21)	0.649 (21)	0.619 (21)	0.623 (1)	1.000 (21)					
P_{100}	0.562 (22)	0.603 (22)	0.591 (22)	0.576 (22)	0.675 (1)	1.000 (22)				
PVR	-0.575 (22)	-0.496 (22)	-0.506 (22)	-0.409 (22)	-0.544 (21)	-0.572 (22)	1.000 (22)			
$\bar{P}_{TA} - \bar{P}_W$	0.103 (22)	-0.181 (22)	-0.411 (22)	-0.443 (22)	-0.487 (21)	-0.639 (22)	0.906 (22)	1.000 (22)		
SV	0.190 (22)	0.311 (22)	0.257 (22)	0.333 (22)	0.410 (21)	0.189 (22)	-0.467 (22)	-0.266 (22)	1.000 (22)	
SaO_2	0.131 (22)	0.06 (2)	0.432 (22)	0.161 (22)	0.442 (21)	0.841 (22)	-0.534 (22)	-0.660 (22)	0.241 (22)	1.000 (22)

number of observations in parentheses

< 0.42% not significant

0.333 > r > 0.499

0.63 > r > 0.134

> 0.632

n = 22

	W _B	TLC	VO	FEV ₁	MVV ₄₀	DLCO	P ₅₀	S ₀ O ₂	SV	PVR	TTB	DV	HR
W _B	1.000 (17)												
TLC	0.182 (17)	1.000 (17)											
VO	0.591 (17)	0.812 (17)	1.000 (17)										
FEV ₁	0.546 (17)	0.685 (17)	0.868 (17)	1.000 (17)									
MVV ₄₀	0.580 (17)	0.821 (17)	0.777 (17)	0.918 (17)	1.000 (17)								
DLCO	0.624 (16)	0.107 (16)	0.130 (16)	0.204 (16)	0.157 (16)	1.000 (16)							
P ₅₀	-0.001 (16)	0.166 (16)	0.376 (16)	0.346 (16)	0.219 (16)	-0.023 (16)	1.000 (16)						
S ₀ O ₂	-0.012 (15)	0.520 (15)	0.218 (15)	0.306 (15)	0.210 (15)	0.198 (15)	0.100 (15)	1.000 (15)					
SV	0.556 (15)	0.556 (15)	0.556 (15)	0.251 (15)	0.142 (15)	0.610 (15)	-0.246 (15)	0.161 (15)	1.000 (15)				
PVR	-0.18 (15)	0.172 (15)	0.179 (15)	0.059 (15)	-0.033 (15)	-0.277 (15)	0.190 (15)	0.611 (15)	0.095 (15)	1.000 (15)			
TTB	0.01 (1)	0.590 (17)	0.431 (17)	0.368 (17)	0.375 (17)	0.686 (16)	0.107 (16)	0.017 (15)	0.450 (15)	0.012 (15)	1.000 (17)		
SV	0.517 (17)	0.574 (17)	0.557 (17)	0.419 (17)	0.411 (1)	0.351 (16)	0.115 (16)	0.164 (15)	0.175 (15)	0.175 (15)	0.78 (17)	1.000 (17)	
HV	0.567 (16)	0.140 (16)	0.243 (16)	0.207 (16)	0.11 (16)	0.184 (16)	0.006 (16)	0.105 (15)	0.590 (15)	0.586 (15)	0.1 (16)	0.419 (16)	1.000 (15)

number of observations (shown in parentheses)

< 0.550 not significant
 0.620 > r > 0.530
 0.776 > r > 0.620
 > 0.776

n = 17

reduction of diffusing capacity. In previous studies it has been shown that the diffusing capacity and the pulmonary capillary blood volume were reduced in patients with pulmonary fibrosis (9). The argument cited above is supported by the positive correlation between W_B , THb , $D_{L_{CO}}$ and SV .

Summary

This study showed that in obstructive patients the low physical working capacity was associated with low ventilatory and diffusing capacities. The latter were found to be most suitable when combined for the assessment of physical working capacity. The coexistence of cardiac dysfunction plays a minor role in the limitation of physical performance. In restrictive patients the low physical working capacity was attributed to the small circulatory dimensions and reduced diffusing capacity.

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CHAPTER VII

GENERAL DISCUSSION

I Pathophysiological changes

Two groups of patients were studied A) 24 patients with obstructive lung disease and B) 17 patients with restrictive lung disease.

The two groups were similar with regard to their anthropometric data and circulatory dimensions (THb BV and HV) (Chapter II)

Physical working capacity

The physical working capacity (Chapters III and VI) measured as W_B was in the obstructive group 52 % of the normal value predicted from the equation given by Irnell and Linder (27) and in the restrictive group 55 %. The cause of interruption of the exercise test in the two groups was dyspnea in about 90 % of cases. Other causes, such as fatigue in the legs, general fatigue and ectopic beats, were recorded for some patients in both groups, either alone or associated with dyspnea. In the *obstructive group* there was a highly significant correlation between the physical working capacity (W_B) on the one hand and the ventilatory capacity and diffusing capacity on the other. The circulatory dimensions and the central circulation were of minor importance for the working capacity compared with the ventilatory or diffusing capacities. This is shown by the weak correlation between W_B and the circulatory dimensions and the data from the measurements of the central circulation.

The present results are in agreement with those from previous studies in obstructive disease, which showed a high relationship between the maximal physical performance, on the one hand, and ventilatory capacity (26 29 34 43) and diffusing capacity (34) on the other.

The working capacity in the *restrictive group* in contrast to the obstructive group, was related neither to lung volumes nor to ventilatory capacity. The significant finding that W_B was highly correlated with the ventilatory capacity in the obstructive group but not in the restrictive group although the ventilatory capacity was practically of the same order in the two groups, suggests that the physical working capacity is not limited by the volume ventilated per se but by other factors associated with airway obstruction. Levison and Cherniack (31) suggested that the oxygen cost of increased ventilation during exercise may be an important factor limiting the work performance in patients with obstructive lung disease. Freedman (18) ascribed the inability to sustain 15 sec MVV for a longer period to the fatigue of the respiratory muscles. The low physical working capacity in the restrictive group was not related to the ventilatory capacity but to the reduced diffusing capacity and to the small circulatory dimensions, presumably caused by the effect of the disease on physical activity.

The physical working capacity (W_B)

in the *obstructive group* was related to circulatory factors and partially to the effect of ventilatory dysfunction. Physical inactivity results in small dimensions of the circulation. The increase in intra thoracic pressure might influence the filling pressures of the heart and results in a small stroke volume. In the *restrictive group* however restrictive ventilation seemed not to have a direct effect on $W_{1/2}$, while circulatory dimensions were important factors. In patients with restricted ventilation due to severe idiopathic scoliosis the low $W_{1/2}$ was ascribed mainly to the reduction in circulatory dimensions rather than to the direct effect of ventilatory limitation (19). In both groups $W_{1/2}$ was slightly correlated with DL_{CO} ($p < 0.05$).

The physical working capacity (W_B) was found to be significantly higher in sitting than in supine position in the two groups. This might be ascribed in both groups mainly to the high airway resistance and work of breathing in recumbent position (10, 35, 37). The working capacity (W_{max}) was also found to be higher in sitting than in supine position in normal elderly subjects (45) while the reverse was found in patients with sarcoidosis who had marked orthostatism during exercise (46).

The respiratory rate at submaximal work load showed wide variations and was not related to the degree of airway obstruction in the obstructive group, but a weak negative correlation was found between respiratory rate at 200 kpm/min and the vital capacity in the restrictive group. It has been claimed that disturbed lung function may be suspected if the respiratory rate increases to more

than 35 breaths/min (33). This seemed probably true in restrictive disease, where high respiratory rates were found in patients with small lung volume.

Ventilation, gas exchange and diffusing capacity

There is a general agreement that arterial hypoxemia in obstructive disease is caused mainly by uneven ventilation/perfusion (28, 35, 43). Although the reports on restrictive disease (pulmonary fibrosis) agree that hypoxemia becomes more accentuated during exercise yet they are conflicting as regards its mechanism (39, 44, 48). The present investigation aimed to elucidate this problem (Chapter IV).

During exercise the alveolar ventilation was more adequate in the restrictive than the obstructive group, as evidenced by the blood gases and by the regression equation of V_A on V_O_2 . The V_D , V_D/V_T , V_A/Q (calculated) and veno-arterial shunt demonstrated a more pronounced inequality of ventilation/perfusion in the obstructive than in the restrictive group. The diffusing capacity was slightly reduced, but identical in the two groups. The $P(A-a)_{O_2}$ was markedly increased in both groups, at rest to a greater extent in the obstructive than in the restrictive group while during exercise $P(A-a)_{O_2}$ was unchanged in the obstructive but increased in the restrictive group. In 11/24 patients in group A and 5/16 patients in group B $P(A-a)_{O_2}$ during exercise was unchanged or decreased.

Piiper (38) in his theory of unequal distribution of diffusing capacity has

demonstrated that when the diffusing capacity is unequally distributed in relation to perfusion the $P(A-a)_O$ would be greater than in a lung with the same diffusing capacity and perfusion but with equal distribution. Applying this concept to the present material the mean ratios V_A/\dot{Q} and $D_{L_{CO}}/\dot{Q}$ were determined in the two groups. As \dot{Q} was not measured at the same time as V_A and $D_{L_{CO}}$ it was calculated by means of the regression equation of the cardiac output on the oxygen uptake obtained during the hemodynamic study (Chapter V). The results showed that during exercise V_A/\dot{Q} was lower in group A than in group B while the reverse applied to $D_{L_{CO}}/\dot{Q}$. Thus, the observed changes in $P(A-a)_O$ and consequently the arterial hypoxemia, were mainly ascribed to uneven ventilation/perfusion in the obstructive group and uneven diffusion/perfusion in the restrictive group.

Central hemodynamics

The effects of hypoxemia on the central hemodynamics of patients with chronic lung disease have been extensively studied by many investigators, particularly in obstructive lung disease. The importance of hypoxemia lies in its intimate relationship with pulmonary hypertension. In the present material the oxygen saturation at rest was slightly reduced in the obstructive group but normal in the restrictive group, and during exercise both groups showed a mild degree of hypoxemia. The present investigation showed in the two groups, at rest and during exercise a normal regression of

pressures on the cardiac output when compared with pressures reported in healthy elderly subjects (21, 22) except for the regression of \bar{P}_{PA} in both groups and RV in group B both of which were slightly significantly above normal. The latter showed a tendency to above-normality in group A. At rest \bar{P}_{PA} averaged 20 mm Hg in both groups compared to 16 mm Hg in normal material ($p < 0.05$). During exercise most pressures displayed a wide individual variation from normal to highly elevated. It is important to point out that the average age in the control material was 70 years compared to 58 years in group A and 54 years in group B so that the pressures in the patient material might probably be higher than in normal subjects of comparable age. The elevation of pulmonary artery pressure was studied in terms of pressure difference between the pulmonary artery mean pressure and the mean pulmonary wedge pressure $\bar{P}_{PA} - \bar{P}_w$ as an increase of wedge pressure contributes to the elevation of pulmonary artery pressure (20, 24). In the two groups the increase in \bar{P}_w during exercise contributed to about 50 % of the increase in pulmonary artery mean pressure. In the obstructive group $\bar{P}_{PA} - \bar{P}_w$ was significantly related to oxygen unsaturation and to increase of carbon dioxide tension. This is in agreement with previous studies, where hypoxemia has been considered the dominating factor in the genesis of pulmonary hypertension (1, 12, 13, 14, 16, 23). Also hyperapnea has been considered an important factor (12, 17, 23, 41). In the restrictive group $\bar{P}_{PA} - \bar{P}_w$ was identical with that in the

obstructive group in spite of the normal arterial blood gases at rest and less marked oxygen unsaturation during exercise. This was ascribed to the effect of reduction of the vascular bed. The present study did not demonstrate any relationship between $\bar{P}_{PA} - \bar{P}_w$ and arterial pH in the two groups. The reports in the literature are conflicting as regards this relationship (cf 1 12, 13 24 41)

The controversy in published reports as to whether cardiac output is normal high or low in obstructive lung disease (4 14 49) has been ascribed to the effect of age on the cardiac output (32). The present investigation showed in both groups almost normal cardiac output in relation to oxygen uptake both at rest and during exercise when the effect of age (5 45) was eliminated.

The present findings are in agreement with those of other investigators who reported normal or almost normal cardiac output at rest and in response to exercise in obstructive lung disease (30 42). Similarly the cardiac output was reported to be normal in patients with pulmonary fibrosis (49). Recently Tammsaara — Hilti (47) reported in patients with severe obstructive lung disease low cardiac output compared to the same normal material as used in the present study. However several of her patients had left ventricular impairment and respiratory insufficiency. Several authors have reported different patterns of cardiovascular dysfunction in obstructive lung disease, e.g. PP and BB patients (15) bronchial and emphysematous type (7) low output and hypoxic pattern (8). The different patterns of abnormality seemed to be due to the difference in

arterial blood gases and to an anatomical destruction of the vascular bed in individual patients, and the difference in published reports was explained by the difference in selection of patients (8). The obstructive group in the present material included a mixed type of bronchitic and emphysematous patients, and the cardiac output was within ± 2 SD i.e. it varied between high normal to low normal. However the causes of this interindividual variation in cardiac output were not analysed in the present study.

The stroke volume increased from rest to exercise by 8 % $p > 0.05$ in the obstructive group and by 23 % $p < 0.01$ in the restrictive group without significant difference between the two groups. Figures of 13 and 16 % have been reported as normal values in healthy young and elderly subjects respectively (11 22). The small increase of stroke volume from rest to exercise in the obstructive group was ascribed mainly to the effect of increased intrathoracic pressure on the filling conditions of the heart.

Effects of obstructive and restrictive ventilatory dysfunction

The effects of obstructive ventilatory dysfunction on the hemodynamics were studied (Chapter V). $\bar{P}_{PA} - \bar{P}_w$ and consequently PVR, were found to increase with increasing degree of airway obstruction, but no effect on the cardiac output was noted. This was mainly caused by the effect of hypoxemia and probably by the increase in intrathoracic pressure.

With increasing degree of restriction there was no effect at rest on the pres-

tures, cardiac output or oxygen saturation. During exercise, on the other hand, it was demonstrated that patients with marked degree of restriction ($VC < 50\%$ of predicted) had \bar{P}_{FA} and \bar{P}_W which were about 7 mm Hg lower than patients with lesser degree of restriction ($VC > 50\%$ of predicted) but $\bar{P}_{FA} - \bar{P}_W$ and PVR were practically identical (Table VI Chapter V). This difference in pressures could not be satisfactorily explained by the difference in age, blood flow, blood volume or blood gases, but was presumably due to a decreased negative intrapleural pressure level.

II. Selection of function tests

1. Physical performance

In patients with *obstructive disease* the physical working capacity expressed as the work load performed at the breaking point of exercise (W_B) was found to be more advantageous than the work load at heart rate 130/min (W_{130}) or the heart rate at the exercise breaking point (HR_B). W_B was found to reflect the degree of severity of disease as evidenced by the high correlation with the indices of ventilatory capacity ($p < 0.001$). Moreover it is always measurable. The disadvantage of using W_{130} was that the work could not be increased to this heart rate in all patients; also an approximate circulatory steady state could not be achieved in some patients. It was weakly correlated to both ventilatory capacity and circulatory dimensions. HR_B , similarly to W_B , was significantly correlated with the ventilatory capacity, but the level of significance was not as high as with W_B . Moreover HR_B might be in-

fluenced by a high resting heart rate which was recorded in several patients in both groups.

In patients with *restrictive disease* neither of the three parameters W_B , W_{130} and HR_B was significantly correlated to the lung volumes or ventilatory capacity. The only correlations found were with circulatory dimensions.

It is suggested therefore, that in patients with obstructive disease W_B is the parameter of choice for assessment of the influence of the respiratory disease on physical working capacity. In restrictive patients, on the other hand, W_B does not reflect the ventilatory impairment.

In patients with lung disease the work test should be performed in sitting position, as both types of patients had a higher work capacity in sitting than in supine position. This is in contrast to other conditions, e.g. in some inactive cardiac patients before chest surgery (25) and in surcoctors (46) in whom orthostatism is marked during exercise and consequently the work pulse is higher in supine than in sitting position.

In the present study W_B was measured as the highest load the patient was able to sustain for 6 minutes. In many instances the patient had to interrupt the work earlier and W_B had to be recalculated (see methods, Chapter I). It may be profitable, therefore, to use exercise periods of shorter duration, e.g. 2–3 minutes, or to use progressive exercise tests (29). A comparison between these methods would be of value.

2. Ventilatory capacity

Spirometry is a simple and one of the most widely used function tests which

provide valuable information as to whether the patient has an obstructive or restrictive ventilatory dysfunction. The relative importance of the total lung capacity and its subdivisions and of the indices of ventilatory capacity for the assessment of physical performance was deduced from the regression analysis of W_B on these variables (Chapter III)

In the *obstructive group* the indices of ventilatory capacity, FVC, FEV₁, MVV₄₀ and MVV_{true}, were highly significantly correlated with W_B ($p < 0.001$). These parameters were more important than the lung volumes or RV/TLC % and FRC/TLC %. The present studies (Chapters III and VI) showed that, by determining W_B and FEV₁ or MVV₄₀, it is possible to decide whether or not the low physical working capacity in a particular patient is due to impairment of the ventilatory function. The ventilatory capacity in obstructive patients was also found to influence other respiratory functions such as $D_{L_{CO}}$, arterial oxygen saturation and the pressure difference across the pulmonary vascular bed.

In the *restrictive group* spirometric data were not correlated with the physical working capacity. In contrast to the obstructive group the functional derangements in this group had generally no causal relationship to restrictive ventilatory dysfunction. Accordingly in this material and within the present range of restriction, the ventilatory tests gave an anatomical measure of restriction but did not reflect a functional derangement.

3 Test of diffusing capacity

The measurement of diffusing capacity

is necessary for the assessment of function in certain lung diseases. However the different methods and techniques used are the cause of wide disparity in the results reported (9)

In the *obstructive group* the steady state exercise $D_{L_{CO}}$ was found to be reduced in 9 of 21 patients. Low values of $D_{L_{CO}}$ were found in patients with low FEV₁ or MVV₄₀ ($p < 0.01$) and high V_D/V_T ratio ($p < 0.001$) which might indirectly signify that $D_{L_{CO}}$ is to a large extent affected by the degree of uneven ventilation. However in the present material emphysema was a dominating disease, so that the reduction in $D_{L_{CO}}$ also reflected a reduction in the available surface area for diffusion. For practical purposes it is difficult, however to quantify the reduction caused by each factor.

Cadigan *et al.* (9) have shown that the low values of D_L steady state compared to D_L single breath in patients with emphysema were due to uneven ventilation and that the patients with severest emphysema had the lowest steady state $D_{L_{CO}}$. Briscoe *et al.* (6) have shown that in emphysema the V_A/Q ratio averaged 2.24 in well ventilated alveoli and 0.23 in poorly ventilated alveoli. The corresponding end-capillary oxygen saturation was 97.5 % and 76 %. They concluded that inhomogeneity of this degree invalidates the calculation of diffusing capacity based on a single mean alveolar CO tension. These factors have caused some authors to condemn the use of the steady state method in obstructive lung disease (3 & 9). The findings in the present investigation suggest, as in earlier reports (2) that in obstructive disease

$D_{L_{CO}}$ steady state is to be looked upon as a test for overall estimation of the oxygen transfer rather than a measure of the diffusing capacity

Thus the measurement of $D_{L_{CO}}$ when combined with FEV_1 or MVV_{40} , was found to increase the precision of prediction of the physical working capacity in these patients (Chapter VI)

In the restrictive group $D_{L_{CO}}$ was reduced in 9 of 16 patients. Although the reduction of $D_{L_{CO}}$ in the restrictive group could explain the marked fall of Pa_{O_2} in some patients, yet a marked fall in Pa_{O_2} was observed also in the presence of normal $D_{L_{CO}}$. It seemed from the present investigation that in restrictive disease the Pa_{O_2} level is more influenced by abnormal regional distribution of diffusion in relation to perfusion than by the impairment of the oxygen transfer across the alveolar capillary membrane per se. The presence of high dead-space and veno-arterial shunt, together with the reduced $D_{L_{CO}}$, in restrictive patients adds to the complexity of the interpretation of diffusing capacity

For the reasons given above, and because the procedure is relatively disagreeable and cannot be applied to severely disabled patients, the measurement of $D_{L_{CO}}$ by the present method seems to be of little value as a routine test. However it is a sensitive test for the assessment of lung function in special circumstances and measures one aspect of lung function that cannot be measured by other methods.

natory as regards the pressure flow relationship. In both the obstructive and restrictive groups the pressures were virtually identical at rest and during exercise. The increase of the pressure gradient across the vascular bed and of the pulmonary vascular resistance was approximately of the same magnitude in the two groups. The regression of cardiac output on oxygen uptake did not differ significantly in the two groups (cf regression equations in Chapter V)

One of the main values of performing heart catheterization in patients with lung disease is to determine whether or not a patient has a normal pulmonary vascular resistance. Being an elaborate investigation, it is therefore important to determine which type of patient is to be catheterized. In obstructive patients the pulmonary vascular resistance was related to airway obstruction. Considering the upper limit of the pulmonary vascular resistance index in our laboratory to be 3 units, it was found that in 10 patients with FEV_1 % lower than 50 % of predicted (subgroup III Table V Chapter V) the mean pulmonary vascular resistance index was increased both at rest and during exercise, while in patients with FEV_1 % between 50—75 % of predicted (subgroup II) the pulmonary vascular resistance was increased during exercise only. It is suggested, therefore, that heart catheterization may be restricted to patients with FEV_1 % lower than 50 % of predicted.

4 Test of cardio-circulatory function

Heart catheterization was not discrimi-

CHAPTER VIII

GENERAL SUMMARY AND CONCLUSIONS

Forty-one patients with chronic lung disease selected among patients referred to this department for functional evaluation were studied. The material was functionally classified into two groups:

- A) 16 men and 11 women, mean age 58 years, with obstructive lung disease and
 B) 12 men and 5 women, mean age 54 years, with restrictive lung disease.

The two groups were similar with regard to age, anthropometric measurements and circulatory dimensions (THb, BV, HV). They had reduced but practically the same ventilatory capacity. The physical working capacity at the breaking point of exercise (W_D) measured as the highest load the patient was able to sustain for 6 minutes, was about 50 % of normal.

The low physical working capacity (W_D) was associated with low ventilatory and diffusing capacities in the obstructive group while in the restrictive group it was associated with small circulatory dimensions and reduced diffusing capacity. Probably these were the main factors limiting work performance in these patients. The central circulation was a minor factor in the limitation of physical performance in the two groups.

The pulmonary gas exchange data in the obstructive group demonstrated a normal average P_{aCO_2} at rest and somewhat elevated during exercise. The arterial P_{O_2} was slightly lower than normal at rest but, in most patients, did not fall during exercise. In the restrictive

group P_{aCO_2} was somewhat lower than in the obstructive group both at rest and during exercise, and the P_{aO_2} was somewhat higher at rest but fell markedly in some patients during exercise. On the basis of the findings of V_D , V_D/V_T , veno-arterial shunt and measured diffusing capacity it was suggested that the arterial hypoxemia is due mainly to uneven ventilation/perfusion in the obstructive group and to uneven diffusion/perfusion in the restrictive group.

The hemodynamic studies demonstrated in the two groups almost normal cardiac output at rest and in response to exercise. The mean pressures at rest were normal except for a slightly elevated pulmonary artery pressure. During exercise most of the pressures showed a wide variation from normal to markedly elevated. The pulmonary vascular resistance was increased in both groups. The wide pressure difference across the pulmonary vascular bed $\bar{P}_{PA} - \bar{P}_{PA}$ was related to the oxygen unsaturation and P_{aCO_2} increase in the obstructive group but less so and non-significantly respectively in the restrictive group. It was not related to the pH in either group.

The stroke volume increased from rest to exercise by 8 % in the obstructive group and 23 % in the restrictive group. The small increase in the obstructive group was ascribed mainly to factors associated with increased intrathoracic pressure.

The pressure difference across the pulmonary vascular bed and the pulmonary vascular resistance increased with increasing airway obstruction but were virtually unchanged with increasing degree of restriction. It seemed from the present investigation that pulmonary hypertension, and consequently cor pulmonale, are consequences of obstructive ventilatory dysfunction. In restrictive patients pulmonary hypertension was also present but was not related to the degree of restrictive ventilatory impairment, and hence other factors might be of importance for the development of cor pulmonale.

It is apparent from the results of the present investigations that alterations in respiratory function have a causal relationship to obstructive ventilatory dysfunction but not to restrictive ventilation. In restrictive disease functional derangements seemed to have a weak relationship to the cause of restriction.

For the evaluation of physical working capacity in obstructive disease, W_{50} was found to be the measure of choice for assessment of the influence of respiratory disease on physical performance. In restrictive disease W_{50} was not related to the ventilatory impairment.

The diffusing capacity and heart catheterization were found in the present study to have a low practical discriminatory value in obstructive and restrictive disease.

Suggested investigation procedure

As screening tests for evaluation of lung function, the exercise tolerance test (W_{50}) and the test of ventilatory capacity are suggested in the first place. When

these tests display a deviation from ordinary values of about 30 % or more, the following investigations are recommended.

A) In obstructive patients measurement of arterial oxygen and carbon dioxide tensions at rest and during exercise.

B) In restrictive patients measurement of arterial blood gases as in A and, if P_{aO_2} displays an abnormal reduction during exercise, determination of diffusing capacity and blood volume are recommended.

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Factors Related to Sudden Death in Acute Ischaemic Heart Disease

A community study in Helsinki

By Matti Romo

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FROM THE RESEARCH DEPARTMENT OF THE FINNISH HEART ASSOCIATION
AND FROM THE FIRST DEPARTMENT OF MEDICINE, UNIVERSITY CENTRAL
HOSPITAL, HELSINKI, FINLAND

FACTORS RELATED TO SUDDEN
DEATH IN ACUTE ISCHAEMIC
HEART DISEASE

A COMMUNITY STUDY IN HELSINKI

by

MATTI ROMO

HELSINKI 1972

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This study is based on material collected by the Ischaemic Heart Disease Register in Helsinki. It is part of an international study co-ordinated by the World Health Organization. The present study was carried out during the years 1969—1972 at the Research Department of the Finnish Heart Association and at the First Department of Medicine, Helsinki University Central Hospital.

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Helsinki, October 1972

Matti Romo

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I INTRODUCTION AND AIM OF THE STUDY

Sudden coronary death (SCD) is without doubt one of the most difficult problems in contemporary medicine. The establishment of coronary care units in the 1960's has contributed a great deal to our understanding of its mechanism. In recent years, mobile coronary care units (MCCU) have drawn ever earlier phases of the acute ischaemic heart disease (AIHD) into the limelight. The essential role of primary arrhythmias was thus pointed out. It has also been found that most of these early arrhythmias can be successfully treated or even prevented if the treatment is started early enough.

However this progress benefits only a minority of all patients with acute ischaemic heart disease at present. It is a fact that two thirds of the total mortality rate in AIHD still consists of deaths outside hospitals. It is natural that the pre-hospitalization phase of acute attacks has attracted increasing interest.

Those who succumb to sudden coronary death in their most active years, often quite unexpectedly comprise a large group among the victims concerned. However it can be noted on closer consideration that many of these have previously suffered from symptomatic atherosclerotic diseases or that in them symptoms have occurred prior to the attack, which simply have not been recognized as being of serious nature.

During the last two decades, the literature of the subject dealing with the risk factors

of ischaemic heart disease (IHD) has increased to a really extravagant degree. The same risk factors which are predisposing to all manifestations of IHD in general seem to be associated with sudden coronary death, although their predictive power may differ in different manifestations of the disease. The factors expressly predisposing to sudden death are only imperfectly known as yet. Additional information is also needed as regards premonitory symptoms and those events which immediately precede sudden fatalities, as well as the circumstances in which they occur. Full understanding of all these aspects is a prerequisite for any programme of prevention or control.

The vital statistics of various countries, together with international comparative studies, have clearly indicated that great differences exist in the occurrence of arteriosclerosis, and of various manifestations of IHD as well as in IHD mortality. It follows that the experiences pertaining to any one country are not as such applicable to another country. In world vital statistics Finland is conspicuous in that it has the highest IHD mortality of males of all countries or regions. Finnish females also range high, although not highest of all. During the most recent years the mortality of IHD has continuously increased in Finland, as it has done in many other developed countries. This alarming trend can hardly be reversed unless attention is more strongly centred on what takes place outside hospitals.

In 1969 an Ischaemic Heart Disease Register was established in Helsinki in co-operation with the World Health Organization (WHO). An attempt has since been under taken to register all cases of AIHD concerning persons aged 85 years or younger within the population of Helsinki.

The Ischaemic Heart Disease Register furnished an opportunity for studying the occurrence of sudden coronary death within an entire community with its total mortality and morbidity of AIHD as a background. It was possible to compare the characteristics of those dying suddenly to those whose death was more delayed and to those who survived their AIHD attack.

The aim of the study was

1 To determine the incidence of sudden

coronary death in Helsinki and to gain a quantitative understanding of its importance.

2 To study demographic characteristics, previous diseases and other risk factors in three comparison groups: sudden coronary deaths (SCD), non-sudden coronary deaths (NSCD) and survivors (SURV) and to assess the possibilities of identifying in advance those persons who are exposed to the highest risk of dying suddenly.

3 To study the prodromal symptoms with regard to pointing out those alarming symptoms, if any which might indicate a mandatory need of intervention.

4 To study the circumstances in which the sudden fatalities occurred in order to reveal potential immediately predisposing factors.

II REVIEW OF THE LITERATURE

The literature concerning various aspects of sudden coronary death has escalated to a large extent. Experimental and clinical studies have served attempts at clarifying the electrophysiological events concerned and their pathological basis in sudden fatalities. Epidemiological studies, on the other hand, have furnished information on the natural history of sudden coronary death in certain communities.

The first part of the present review is dedicated to the definition of sudden coronary death. The pathological basis of sudden coronary death and its mechanism are also briefly considered. Some prominent epidemiological studies concerning sudden coronary death in different countries are reviewed. Ultimately certain characteristics, previous diseases and other risk factors related to sudden fatalities are discussed.

1 DEFINITION OF SUDDEN CORONARY DEATH

A major difficulty in comparing and interpreting the results of previous studies on sudden death has been lack of a uniformly accepted definition (Kuller 1966). Nearly all studies exclude violent death, such as suicide, accidental death and poisoning. However there is disagreement as to what should be the duration of the fatal attack and as regards its unexpected nature, which has also been used as a criterion by many authors.

In 1959 a WHO expert committee found that at the time no sufficient evidence existed to justify the use of sudden death as an index for coronary heart disease. It

was stated, however that it could become one if more information were gathered on its occurrence in an unselected population and on its various causes, such as pulmonary embolism, cerebral accidents and dissecting aneurysm on the side of coronary heart disease (Hypertension and coronary heart disease 1959). The committee suggested that the term sudden death should be employed to mean a death occurring instantaneously or within a few minutes.

In 1969 an expert committee supported by the Scientific Council on Arteriosclerosis and Ischaemic Heart Disease of the International Society of Cardiology and by the Councils on Arteriosclerosis and Epidemiology of the American Heart Association suggested the following definition. Sudden unexpected death is a death occurring instantaneously or within an estimated 24 hours of the onset of acute symptoms and signs. The suggestion also included a proposal that sudden deaths should be further subdivided into those occurring within one hour 1-12 hours and 12-24 hours, respectively.

In spite of these recommendations, the time interval used in various studies differs from 15 minutes to 24 hours and even longer. In some studies no time limit whatsoever is applied instead, the place of death or the unexpected nature of the death have served as a criterion for selection. Deaths in hospitals or other institutions are often excluded from studies concerning sudden death. The practice varies as regards unwitnessed deaths with unknown duration of the fatal attack.

Other more serious difficulties arise when

It is desired to define the concept of sudden coronary death. Even if a definite time limit were specified for the fatal attack and the unexpectedness of the event were precisely defined, the difficulty remains that, even in autopsy it is often difficult to demonstrate the cause of sudden death with certainty. There are no generally accepted criteria for autopsy findings which might justify entry of AIHD as a cause of death. The pathologist can, however rule out other reasons for sudden death and furnish indirect evidence for AIHD as being the most probable cause of death. It is further noted that autopsy is infrequent in many communities. In many population studies having a low autopsy rate sudden deaths have been attributed to AIHD if no other obvious cause of death can be pointed to.

2. ISCHAEMIC HEART DISEASE AS A CAUSE OF SUDDEN DEATH

The references to different causes of sudden death in numerous text-books on forensic medicine are mainly based on medico-legal autopsy series. These series may be biased but, all the same, the essential role played by IHD as a cause of sudden death becomes clearly evident in nearly all autopsy studies. Diseases of heart and aorta are considered responsible for 45 to 85 per cent of sudden deaths. The largest contribution in this group is that of IHD varying from 50 to 79 per cent of the group mentioned (Kuller 1966 Uotila 1970).

In most series of medico-legal autopsies no differentiation is made with regard to duration of the terminal event. Such studies exist, however which suggest that the role of IHD as a cause of sudden death becomes even more prominent when sudden death is defined in terms of a comparatively short time interval. In a study by Spain et al. (1960), the material concerning 1329 consecutive autopsies was divided into three

categories according to the duration of the fatal episode less than one hour from one to three hours and undetermined time (unwitnessed death). The authors showed that in the first category 91 per cent of the deaths of males, but only 52 per cent of those of females, were due to IHD. In the second category the proportion of IHD was less than 85 per cent, and in the third category 61 per cent in males and only 35 per cent in females. Similar results were noted in a cross-sectional study of sudden death in Baltimore (Kuller et al. 1967). In the age group of 40 to 64 years 91 per cent of the deaths of white males occurring within two hours were due to IHD or hypertensive heart disease the corresponding figure for white females was 95 per cent. In contrast, only 69 per cent of white males' and 89 per cent of white females' deaths occurring between 2 and 24 hours after the onset of symptoms were due to these diseases. In the group of unwitnessed deaths the figures were 79 and 64 per cent, respectively.

The figures stated above are in good agreement with the current understanding of the mechanism of sudden coronary death. Cardiac arrest causes death within a few minutes, while the duration is somewhat longer with other mechanisms of natural death. Unwitnessed deaths constitute a heterogeneous group of cases, with variable duration of the fatal event and probably also differing in other respects from the group of the witnessed deaths.

3 MECHANISM OF SUDDEN CORONARY DEATH

One of the major benefits from coronary care units has been that better clarification of the mechanism of sudden coronary death has been attained and the great importance of the early arrhythmias has been revealed.

Hellerstein and Turell (1964) reported that ventricular asystole was the commonest

terminal electrocardiographic finding in a series of hospitalized patients who succumbed to sudden death. However most investigators have shown that ventricular fibrillation is more common (Lown et al. 1967 Crook 1968, Adgey et al. 1969 Dixon 1970). This is also in agreement with animal experiments (Carroll et al. 1965).

Many reports, e.g. from Belfast, have pointed out the high frequency of rhythm disturbances at the very beginning of an acute attack. Patients arriving at hospital have very often already passed the most dangerous phase of their acute illness and the rhythm disturbance pattern has also changed. In a recent report from Belfast (Adgey et al. 1971), based on patients who were reached by a mobile coronary care unit (MCCU) within one hour of the onset of an AIHD attack, no less than 233 of 284 patients (82 per cent) had some kind of arrhythmia, and in 170 patients dysrhythmia appeared within the first hour and required therapy before the patient was considered fit for transport to hospital. Brady cardia was highly common during the first hour (in 31 per cent) and it has been shown as enhancing the ectopic ventricular activity (Han 1969). Hypoxia with all the consequences attendant upon it, together with the electrical instability of the myocardial tissue, creates conditions conducive to ventricular fibrillation. Primary ventricular fibrillation occurred in 5.5 per cent of patients admitted to a coronary care unit within 4 hours after the onset of symptoms, as contrasted with an incidence of 0.4 per cent when admission was delayed (Lawrie et al. 1968). According to Pantridge and Geddes (1967) ventricular fibrillation is 25 times more frequent during the initial 4 hours than during the remaining part of the first 24 hours.

Many authors have reviewed the topic (Surawicz and Pellegrino 1964, Lovell and Princes 1971, Lown and Wolf 1971 James 1972).

4. PATHO-ANATOMICAL FINDINGS

The morphological changes in myocardial infarction are not visible by conventional methods until some six hours have passed after onset of the process. It is, therefore, natural that if sudden death is defined as one occurring within one hour these changes are usually absent. It is peculiar that in most studies a certain proportion of cases displayed pathological changes, suggesting a myocardial infarction. In the series of sudden coronary deaths reported the frequency of fresh myocardial infarction has usually varied from 8 to 24 per cent (Adelson and Hoffman 1961 Franco 1962, Spiekerman et al. 1962, Hansen 1968 Luke and Helpern 1968, Wikland 1971), but even a higher frequency (47 %) is recently reported (Scott and Briggs 1972).

In many cases of sudden death attributed to IHD the sole finding is diffuse coronary atherosclerosis, often more advanced than could be expected on the basis of age. Patches of myocardial fibrosis and scars after an old infarction are also common, and the number of scars usually exceeds that of the clinically recognized episodes (Slevers 1963, Wikland 1971).

The frequency of fresh coronary thrombosis varies in different studies from 15 to 89 per cent (Croce et al. 1960, Crawford et al. 1961 Franco 1962, Hallerman 1962, Mitchell and Schwartz 1965 Hansen 1968, Kagan et al. 1968, Luke and Helpern 1968, Spain et al. 1969 Rissanen 1970 Titus et al. 1970 Scott and Briggs 1972). Many reasons have been speculatively suggested for this variability including differences in autopsy technique and differences between the populations studied and in definitions of sudden death. On the whole, however it can be said that the occurrence of a fresh thrombus in the coronary arteries seems to be rarer in cases of sudden death than in cases with a more delayed yet eventually fatal course. Spain and Braden (1960 and 1970) claim that the

frequency of thrombosis correlates with the patient's survival period, increasing with a longer duration of the attack. They also dispute the common idea that myocardial infarction is preceded by thrombotic occlusion on the contrary they suggest that thrombosis may be a sequela of myocardial infarction. This contention has received emphatic support from Baroldi (1965). Meadows (1965) and Kagan et al. (1968), too, have failed to find evidence in favour of the traditional idea that an occlusion of a main coronary artery has to occur before myocardial infarction develops.

Spain et al. (1963) also stress the importance of collateral circulation, and they claim that lack of such circulation may be an essential cause of sudden death. In contrast, Baroldi (1969) also demonstrated an extensive collateral network in patients who had died suddenly without any preceding symptoms indicative of coronary heart disease. He states that occlusion of coronary arteries is of less importance in the development of myocardial infarction, which originates in the cells of the myocardium.

James has emphasized the need for careful studies of the small coronary arteries (1967), the blood supply of the sinus node (1966) and the histology of the conduction system (1969). Lesions of the conduction tissue in cases of sudden death have also been reported by Lamb and Shacklett (1982).

It can be stated that, at present, it is often impossible in autopsy to demonstrate by conventional technique the cause of sudden death with certainty (Edwards 1971, Schwartz and Walsh 1971). The high prevalence of coronary atherosclerosis in our adult population further impedes the determination of the cause of sudden death. Microscopical examination improves the accuracy of diagnosis to a certain degree and the same is achieved by more recent histochemical and other methods (The Pathological Diagnosis of Acute Ischaemic Heart Disease

1970). Their value and usefulness in practice remains to be demonstrated.

5 POPULATION STUDIES

Apart from autopsy studies, several cross-sectional or retrospective as well as prospective studies performed both in U.S.A. and in Europe have aimed at determining the frequency of sudden coronary death in a given specific population.

U.S.A.

Spiekerman et al. (1962) studied all deaths in Rochester Minnesota, a small community with a population of 30 000 having an exceptionally high autopsy rate in the United States during the five-year study period, 1947 to 1951. There were altogether 1026 deaths at the age of 20 years or older of which 691 (67 per cent) were autopsied. Of these patients 221 had died of IHD and 114 of these deaths (52 per cent) were sudden. No definition of sudden death was given.

The Health Insurance Plan (H.I.P.) of Greater New York Study comprised 55 000 men aged 25–64 years. During a four year period beginning in 1961 851 men experienced their first myocardial infarction or sudden death. Sudden death, defined in the study as one occurring within 24 hours, accounted for 271 (30.6 per cent) of these first events (Shapiro et al. 1965, Weinblatt et al. 1968).

An extensive community study of sudden death was carried out in Baltimore in 1964–1965 (Kuller et al. 1966 a, b, c, 1967, Kuller 1969). Sudden death was defined as a death occurring within 24 hours and concerning a person not restricted to his home, to a hospital or to another institution and not unable to function in the community over more than 24 hours prior to death. 60 per cent of all IHD deaths occurred within 24 hours and 61.4 per cent of all sudden deaths were due to AIHD.

Several other community studies have revealed closely similar figures as those mentioned above (Zukel et al. 1959; Eisenberg et al. 1961; Balinton and Peterson 1963).

In addition to the community studies mentioned, there are also several prospective epidemiological studies, many of them with a large cohort and a long follow-up period, but even so these are hampered by a relatively small number of sudden deaths.

Perhaps the most notorious of them all is the Framingham Study in which results from a 12 and 14 year follow-up period have been reported (Kannel et al. 1966; Gordon and Kannel 1971). In a cohort of 5209 persons, 120 died of IHD during 14 years, and 88 of these deaths (55 per cent) occurred within one hour.

In the Tecumseh Study 98 deaths due to IHD were noted during the six year period from 1959 to 1965 and 45 of them (46 per cent) occurred within one hour (Chiang et al. 1970).

Some preliminary data are available from the national co-operative pooling project of the U.S.A., which combines the results of seven extensive prospective cardiovascular studies (Stamler 1971). Of 7394 men aged 30–59 and free of IHD at entry 601 experienced a first event of AIHD during the ten-year observation period. 123 (24.6 per cent) of these events were sudden deaths, defined as a death occurring within three hours from the onset of illness.

The studies mentioned above as well as several other prospective studies (Doyle et al. 1959; Fell and d'Alonzo 1964; Stamler 1967), have altogether consistently indicated that sudden death occurs, as the first manifestation of IHD in 20–25 per cent of cases.

Europe

Studies reported from England and Northern Ireland have produced closely similar results to comparable studies in the U.S.A.

Fry studied in his own practice, in London,

the incidence of myocardial infarction and sudden death over a period of 17 years, from 1949 to 1966. In a population of 5500 persons on the average, he found 350 new cases of IHD and 149 of them terminated fatally. 97 deaths occurred within 24 hours and 80 deaths within one hour (54.4 per cent of all AIHD deaths) (Fry and Dillane 1967).

In Stratford-on-Avon, McWhinney (1968) studied the same problem in an extensive group practice. 28 of 131 events (21.5 per cent) observed over a five-year period were sudden deaths.

All deaths attributed to IHD were studied in one year 1965–1966 in Belfast, Northern Ireland (McNelly 1967; McNelly and Pemberton 1968). Various time intervals, including that from onset of symptoms to death, were carefully investigated. Altogether 998 deaths due to IHD were recorded. 352 of them (33 per cent) occurred within the first hour. In 127 cases the time interval was not known.

In the Edinburgh Community Study in 1967–1968 all cases of AIHD in people under 70 years were registered (Armstrong 1968; Fulton et al. 1969; Armstrong et al. 1972). This study has been a model for the ischaemic heart disease registers in other countries. The proportion of sudden deaths of all IHD deaths closely paralleled the figures from Belfast. In one year altogether 1298 cases of AIHD were registered, and 42 per cent of them died during the first four weeks. Of these deaths 35.4 per cent occurred within one hour and 58.2 per cent within 24 hours. Deaths within one hour constituted 14.8 per cent of all AIHD cases.

Some data exist concerning IHD deaths outside hospitals in the Scandinavian countries, too.

In Denmark an attempt was made to elicit the morbidity and mortality of myocardial infarction over two months throughout the whole country (Moebech and Dreyer 1965). The study was based on notified cases from the hospitals' departments of medicine and

on death certificates covering the same two-month period. Altogether there were 1530 deaths in IHD of which 872 (57 per cent) occurred outside hospital.

In Gothenburg Sweden, the local Ischaemic Heart Disease Register started a pre-pilot study in November 1968. So far only results of this pre-pilot study of a three month duration have been published (Fodor 1969). During this period 470 cases of AIHD were registered in all, 166 of them concerning persons under 66 years. 63 of these 166 patients died, 36 outside hospital (i.e. 22 per cent of all AIHD cases and 57 per cent of all AIHD deaths).

Recently Wiklund (1971) has studied all medically unattended deaths in Stockholm from IHD over a one-year period. This material of unattended deaths consists of 967 deaths which occurred outside hospitals or other institutions for the aged or chronically ill. 278 of the subjects were under the age of 65. Incidence rates for different age and sex groups are given. Attempts were also made to determine the duration of the fatal attack. This could be done in 595 cases. In 413 cases (69.7 per cent of fatal attacks with known duration) the death occurred within one hour. The prevalence of various previous diseases is presented. An analysis of different causes of sudden death other than IHD was made concerning the same period.

Finland

Information about IHD mortality in Finland is available from the official vital statistics, but data concerning sudden coronary death are scanty.

In Espoo, a neighbouring town of Helsinki, Gorbатов et al. (1969) investigated all cases of myocardial infarction in 1963. The study was based on hospital records and death certificates. In a population of 77 341 164 events altogether were found during the

study year 111 in males and 53 in females. 87 persons died (53 per cent of registered cases) 72 of them (83 per cent of all deaths) outside hospital.

A prospective epidemiological study has been in progress in two different areas in Finland since 1959 as part of an international co-operative study undertaken in seven countries (Karvonen et al. 1970 Keys 1970). A cohort of 1711 men aged 40 to 59 years and free of IHD at entry have been under observation for ten years. 101 IHD deaths were noted. The duration of the fatal attack was known in 88 cases. It was less than one hour in 45 cases (51.6 per cent of fatal attacks with known duration) (Punsar 1971).

Several studies seem to suggest that the IHD mortality and hospital admission rate for AIHD have been increasing in Helsinki (Uotila 1945 Varpela 1960 Gorbатов 1961 Sipilä 1966 Tenhu et al. 1970 a, b Uotila 1970 Gorbатов et al. 1971).

In an extensive autopsy series, consisting of all medico-legal autopsies performed at the Department of Forensic Medicine of the University of Helsinki 1923-1942, Uotila (1945) reports that diseases of the heart and aorta were responsible for 59.5 per cent of all these deaths, while IHD constituted 62.9 per cent of these diseases. The corresponding figures in material from the years 1966-1967 of the same department were 85 and 79 respectively (Uotila 1970).

During the years 1959-1968 altogether 3044 cases of AIHD in which death occurred within 24 hours were autopsied at the same department. In this series, deaths within one hour constituted 64 per cent of male and 55 per cent of female cases. 17 per cent of the males and 30 per cent of the females were found dead and the accurate duration of the fatal attack could not be defined. In only 19 per cent of the males and 21 per cent of the females death occurred during the remaining 23 hours (Tenhu et al. 1970 a).

6 FACTORS RELATED TO SUDDEN CORONARY DEATH

During the two most recent decades the literature dealing with the risk factors of IHD has expanded to be voluminous and many extensive reviews have been presented (Epstein 1965 Stamler 1967 Simborg 1970).

Marital status

Studies concerning the possible relationship of marital status to IHD have yielded contradictory results. Most of them have perhaps tended to suggest a certain over-mortality among non-married, in particular widowed persons (Kraus and Littenfeld 1959 Zalokar 1960 Sheps 1961 Bainton and Peterson 1963, Rees and Lutkins 1967 Parkes et al. 1969). In Belfast, the IHD mortality in the age groups between 40 and 69 years was significantly higher for widowed than for married or for single persons (McNeilly 1967). No relationship between marital status and duration of the fatal attack could be found, however.

There are also several studies which have failed to suggest any association between marital status and IHD mortality: they include the prospective studies of Framingham (Kannel et al. 1957a) and Tecumseh (Chiang et al. 1970) as well as the HLP study in New York (Shapiro et al. 1969) and the study of Yater et al. (1948).

Social class

The association between social class and IHD has been an object of lively investigation during recent decades (Marks 1967 Antonovsky 1968 Hinkle et al. 1968). Earlier reports seemed to show quite consistently higher incidence of IHD in the higher social classes. In recent years the picture has become rather more obscure, and several reports state an inverse correlation.

Data on the relationship between social

class and sudden coronary death are scarce. No correlation was found in Belfast by McNeilly (1967) between social class and occupation on one hand and the duration of the fatal attack of AIHD on the other.

In Finland, Sipilä (1966) noted with regard to hospital patients with myocardial infarction in Helsinki that the distribution of social classes paralleled that of the population on the whole. In Espoo, Gorbатов and coworkers found a higher morbidity and mortality of IHD in the lower social classes (Gorbатов et al. 1969). In a study of the IHD mortality in Helsinki, based on death certificates, Gorbатов et al. (1971) observed the highest mortality in the lowest social class, in which the increase in mortality after 1955 was also most striking.

Previous ischaemic heart disease

The most indisputable predictor of ultimate death and sudden death from IHD is the symptomatic IHD itself. Several studies have demonstrated the continuously high mortality following a myocardial infarction. Approximately 20 per cent of middle-aged men who survive an initial attack of myocardial infarction die during the following five years (Weinblatt et al. 1968 Stamler et al. 1969), a substantial proportion of them suddenly. The mortality is highest during the first six months after the attack, but it remains 5 to 10 times that of the general population for several years (Hagström et al. 1967 Weinblatt et al. 1968 Stamler et al. 1969 Zukal et al. 1969 Chiang et al. 1970, Stamler 1971). The mortality in recurrent attacks is higher than that in initial attacks, although the mode of death in recurrent attacks seems to be delayed rather than sudden (Achor et al. 1956, Weiss 1956 Liebow and Badger 1963, Hagström et al. 1967 Badger and Liebow 1968 Rissanen et al. 1971).

The elevated mortality of patients with angina pectoris is also shown in several studies (Seim 1960, Frank 1968, Weinblatt

et al. 1968, Chiang et al. 1970 Kannel and Feinleib 1970 and 1972, Gordon and Kannel 1971) Angina pectoris combined with hypertension or with an abnormal electrocardiogram is of particular importance in increasing the fatal risk (Seim 1960 Frank 1968 Oxman et al. 1970 Punar 1972) In Seim's series of patients with angina pectoris, 37 per cent of IHD deaths were sudden, a figure which, however is rather lower than the frequencies of sudden death usually reported in an unselected population.

Diabetes mellitus

The atherogenic effect of diabetes mellitus is well known (e.g. Bradley and Partamian 1963 Robertson and Strong 1968) Evidence of IHD has been demonstrated in half of diabetics over the age of 40 (Bryfogle and Bradley 1957) Premature IHD even at a relatively young age is not infrequent in diabetics.

On the other hand, a significantly elevated prevalence of diabetes mellitus or abnormal glucose tolerance has been demonstrated in patients with various manifestations of IHD (Ostrander et al. 1963 Epstein 1969) An outstanding feature of diabetic myocardial infarction patients seems to be their rather common lack of chest pain as an initial symptom (Bradley and Schonfeld 1962).

In the Framingham as well as the Tecumseh study diabetes mellitus seems to be associated in particular with death in AIHD (Kannel et al. 1967 a, Epstein and Ostrander 1971).

Hypertension

The relationship of elevated blood pressure and IHD is well established (Deming 1968) As an atherogenic factor hypertension increases the incidence of all manifestations of IHD and very distinctly its mortality (Robertson and Strong 1968 Weinblatt et al. 1968 Oberman et al. 1969) Therefore its fre-

quency among those who die suddenly is also naturally high. This has been reported in e.g. the Framingham (Kannel et al. 1971) and Tecumseh (Chiang et al. 1970) studies. In fact, the figures from the Framingham study seem to indicate that hypertension has a stronger association with the incidence of sudden death than with any other manifestation of IHD (Dawber and Thomas 1971).

On the other hand, in Baltimore the prevalence of hypertension was higher among those whose death was not sudden compared to those who died suddenly (Kuller et al. 1966 a). Neither could Wikland (1971) in Stockholm find any relation between the history of hypertension and the duration of the fatal attack.

Obesity

Contradictory results have been reported concerning the relationship of obesity and incidence of IHD (Seltzer 1969) A positive correlation was demonstrated e.g. by Master et al. (1953) Master and Jaffe (1955) Doyle et al. (1959), Marks (1960), Stamler et al. (1960) Shapiro et al. (1969). On the other hand, e.g. Yater et al. (1948), Keys (1954), Sanders (1959) Paul et al. (1963) and Pell and D Alonzo (1964) found no distinct correlation.

More recently prospective epidemiological studies have revealed that obesity like many other risk factors, bears different relations to different manifestations of IHD The Framingham study has shown that obesity adds to the risk of angina pectoris and of sudden death, but not to the incidence of myocardial infarction (Kannel et al. 1967 a and b) Those with a relative weight of more than 20 per cent above the median had a marked excess risk of sudden death. Later on, data from the Tecumseh study have confirmed the association of obesity with SCD (Chiang et al. 1970) On the other hand, no excess prevalence of overweight was found in three autopsy series of sudden

coronary death (Croce et al. 1960 Adelson 1961 Hansen 1968) No association between obesity and autopsy finding of coronary atherosclerosis appeared in the International Arteriosclerosis Project (Montenegro and Solberg 1968)

Smoking habit

Numerous epidemiological studies have quite consistently reported a correlation between smoking habit and incidence of IHD and the exceptions are few (Zukel et al. 1959 Doyle et al. 1962, Bainton and Peterson 1963 Paul et al. 1963 Doyle et al. 1964, Mulcahy and Hickey 1966 Kannel et al. 1967 a, Shapiro et al. 1969 Punar and Pyörälä 1970) Cigarette smoking seems to be associated with an increased incidence of myocardial infarction and of sudden coronary death, while the connection with angina pectoris has remained uncertain. The risk correlates with the number of cigarettes smoked daily but hardly with the duration of the smoking habit. Pipe and cigar smokers run a relatively small risk, which is close to that of non-smokers. Discontinuation of cigarette smoking results in decrease of both morbidity and mortality

The effect of smoking on the development of IHD is most prominently seen in the young age groups, and it loses much of its strength after the age of 55 years. Many studies, though not all, suggest that cigarette smoking promotes atherosclerosis of the coronary arteries (Auerbach et al. 1963 Strong et al. 1968 Rissanen et al. 1972) as well as other arteries (Auerbach et al. 1968, Sackett et al. 1968)

The mechanism by which smoking exerts its effect is not yet understood though several possibilities exist (Oram 1968 Kjeldsen 1969). It seems likely that, in addition to its potential atherogenic effect, smoking has other more immediate and so far unknown, modes of action. It has been observed that in dogs exposed to cigarette smoke the threshold for

ventricular fibrillation becomes subnormal for periods of up to one hour (Webb et al. 1968).

The experience gained in the Framingham study suggests a very distinct relationship between smoking and sudden coronary death (Kannel et al. 1966 1967 a) This association is most clear-cut in heavy smokers, who have a fivefold excess risk of dying suddenly compared to non-smokers. Sudden death was also a common occurrence among smokers in the autopsy series of Spain et al. (1969) and of Spain and Braden (1970) as well as in an autopsy series of the Ischaemic Heart Disease Register in Helsinki during the pre-pilot period 1969 (Rissanen et al. 1971) Contradictory results have been reported from the Tecumseh study where those who died suddenly had smoking habits equal to those of the population at large (Chiang et al. 1970).

Physical activity

Since the classical work of Morris et al. (1953) was published, the association of physical activity and IHD has been the object of numerous epidemiological studies (Brown et al. 1957 Chapman et al. 1957 Zukel et al. 1959 Brunner and Manella 1960 Stamler et al. 1960, Paul et al. 1963, McDonough et al. 1965, Frank et al. 1968, Kannel 1967) In some respects, the results have been contradictory although most of them seem to show an inverse correlation between the level of physical activity and the incidence of IHD At least two outstanding studies in the U.S.A., the HLP Study in New York and the Framingham Study have indicated that the association of physical activity with the occurrence of sudden death is most strongly in evidence (Shapiro et al. 1969 Dawber and Thomas 1971).

Most of the previous studies are based on occupational physical activity only However Frank et al. (1968), Kannel (1967) and

Tibblin (1970) among others, have shown the importance of off job activity on the side of on-job activity

While most studies have suggested an association between the level of physical activity and the incidence of myocardial infarction and mortality in IHD its correlation with angina pectoris has remained dubious.

It is not understood how exercise may be able to guard against myocardial infarction and coronary death. In animal experiments, an increase of collateral circulation has been shown (Eckstein 1957). In a large autopsy series, Morris and Crawford (1958) reported fewer fibrous patches and myocardial scars and also slightly fewer occlusions of the coronary arteries among those with active occupations compared to those engaged in sedentary occupations.

Other risk factors

Nearly all prospective epidemiological studies have indicated a strong correlation between the level of serum cholesterol and the incidence of all manifestations of IHD. This becomes clearly evident e.g. in the Framingham study (Kannel et al. 1966 and 1969). Relationship of IHD with serum tri glycerides has also been shown (Albrink and Man 1959 Brown et al. 1963), although this association seems to be weaker than that concerning cholesterol.

A familial aggregation of IHD is shown in many studies (Epstein 1964, Rose 1964, Slack and Evans 1966, Oscherwitz et al. 1968) and the significance of genetic factors both independently and through several confirmed risk factors seems to be established.

Several studies have suggested the importance of the personality pattern and of various psychological characteristics for the development of IHD (Friedman and Rosenman 1960 Friedman 1964 Ostfeld et al. 1964

Rosenman et al. 1964, Keith 1966 Rosenman et al. 1967 b Wolf 1969)

Rahe and Lind (1971) in Stockholm gathered data of the changes in life of 39 patients, from a time period covering three years before their sudden death. A significant increase in the subjects' life change intensities during the final six months was noted compared with chronologically equivalent time periods 2 and 3 years earlier. So far unpublished data from a study in Helsinki, using the same methodology have come to similar results (Rahe et al. 1972)

Prospective studies have suggested the importance of certain electrocardiographic changes in predicting the group at highest risk to die suddenly. Definite evidence of left ventricular hypertrophy carried a very high risk in the Framingham study similar to that of patients who have survived a myocardial infarction (Kannel et al. 1969 Kannel et al. 1970 Gordon and Kannel 1971)

Conduction defects, bundle branch block, and certain disturbances of rhythm, especially ventricular premature beats, have all been demonstrated to constitute an excess risk of dying suddenly (Chiang et al. 1969 Lown et al. 1969 b, Lovell and Princeas 1971 Lown and Wolf 1971)

Numerous other risk factors, which are more or less mutually connected, have been suggested.

A report from Canada has suggested that the hardness of household water might be a factor predisposing in particular to sudden death (Anderson et al. 1969). Results on the same line have later been reported from the U.S.A. as well (Peterson et al. 1970)

The prevalence of the risk factors mentioned above is great in a normal population and various combinations of risk factors are present in numerous individuals. While many risk factors have an independent effect the combinations obviously multiply the risk.

III MATERIAL AND METHODS

1 STUDY POPULATION AND AREA

The City of Helsinki

Helsinki is the capital of Finland, and also its largest city. It is located on the southern coast of the country. The area of the city is 176.9 km². The length in the north-south direction is 17.5 km and in the east-west direction 18.6 km. Fig. 1. is the map showing the area of the city in it the hospitals with an emergency reception for patients suffering from heart attacks have been indicated.

Being the capital, Helsinki is the centre of administration, but trade, industry and education are also well represented.

The annual mean temperature in Helsinki in 1970 was 4.0 C, the highest 28.8 and the lowest -28.3. The mean relative humidity was 80 per cent.

The average population of Helsinki during

Table 1 Mean population of Helsinki by age and sex in the year 1970

Age	Males	Females	Total
—19	69337	69009	138346
20—24	26415	23311	89726
25—29	24604	27160	51764
30—34	18736	19636	38372
35—39	15821	16718	32539
40—44	15156	16810	31966
45—49	13353	17692	31035
50—54	11479	16521	28000
55—59	12671	16403	29073
60—64	10524	17823	28337
65—69	7150	13829	20979
70—74	4260	10346	14606
75—	3700	12475	16175
	232587	290241	522828

the year 1970 was 522 928 (Table 1). This implies that about 12 per cent of the population of Finland resided in Helsinki in 1970. During the most recent years the total city population has increased by about one per cent yearly but the figure has remained unchanged or even decreased in some middle-aged groups: one of them — the 50—59 age group during the last five years. This is due to the fact that middle-aged people emigrate in considerable numbers, mainly to the rapidly growing, modern communities adjacent to Helsinki.

Health Services

In 1970 there were 1716 physicians in Helsinki, not all of them in practice. This is more than a third of all physicians in Finland.

In Finland it is the rule to send a patient to a hospital whenever suspicion of a myocardial infarction arises. The few exceptions are those cases where diagnosis is not made before the acute phase of the attack has passed.

Altogether 11 hospitals with departments of medicine have facilities for the care of patients with myocardial infarction. The largest is the Mellahti Hospital of the University Central Hospital, which comprises three separate medical departments. The fourth medical department of the University Central Hospital has a separate location. The city of Helsinki owns four hospitals which have a medical department. Kivela, Maria, Malmi and Aurora Hospitals, furthermore a large hospital for the chronically ill (mainly geriatric) patients. There are also four

private hospitals, one Military Central Hospital and a ward at the Institute of Occupational Health, each of which can potentially take care of myocardial infarction patients.

An emergency reception open day and night can be found in only four of the hospitals: Mellahti, Kivellä, Maria and Malmi (Fig. 1). These hospitals treat more than 90 per cent of the hospitalized myocardial infarction patients in the city for obvious reasons. As can be seen from Fig. 1 three of these four hospitals are located quite close to each other in the centre of the city; only the smallest hospital, that of Malmi, lies in the north-eastern part of the city.

Only two hospitals have a coronary care unit. In the Mellahti hospital, up to eight beds were reserved for coronary patients, and four in the Kivellä hospital.

There is an Alarm Centre providing doctors for house calls around the clock. After office hours five doctors as a rule are available for home visits in different

parts of the city. Telephone calls made to the Alarm Centre are answered by a nurse, who has authority to dispatch an ambulance for transport or who may advise immediate transportation of the patient to a hospital. Home visits are usually made in the order of the calls received, but it has been a rule to give priority to patients suspected of heart attack. There was no direct contact by radio telephone with the cars of the doctors on duty; therefore immediate contact with them at any time has not been possible. At normal times the Alarm Centre arranges for approximately 100 house calls daily.

The ambulance service in Helsinki is organized in connection with the Fire Service of the city. The ambulances are stationed at four different points, but with a joint telephone exchange. As a rule, a doctor's or nurse's authorization is required for any ambulance transport, but in acute situations, which specification is met by patients suspected of suffering from a heart attack,

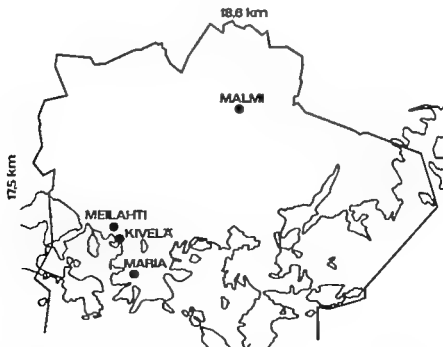


Fig. 1 Map, showing the extent of the city of Helsinki area and locations of the four hospitals having an emergency reception.

urgent transportation is carried out without authorization by any member of the medical profession. In addition to the municipal system, there are several private ambulance companies, some of them also on call around the clock.

Finland has a national health insurance scheme, and all persons under the age of 65 receive a per diem allowance if a doctor prescribes sick leave for longer than 7 week days provided that they are not already recipients of a disability pension.

2. THE ISCHAEMIC HEART DISEASE REGISTER

General outlines of the study

An Ischaemic Heart Disease Register was established in Helsinki, starting September 1st, 1969 as a part of the research programme of the Finnish Heart Association (Siltanen 1973). The design of the study and the outlines and principles followed are those proposed by the World Health Organization and consistent with other ischaemic heart disease registers in various countries, incorporated in the WHO ischaemic heart disease register programme. The study programme is documented in a series of reports prepared by working groups convened by the Regional Office for Europe of WHO (Working Group of Ischaemic Heart Disease Registers, EURO 5010 (1) 1968, (2), 1969, (4), 1970 and (3), 1971). From the very beginning there has been close co-operation with the Regional Office for Europe of the World Health Organization.

The aim of the project is to collect more and better information concerning the most severe forms of acute ischaemic heart disease, myocardial infarction and sudden death as they occur throughout the community. According to the recommendation of WHO any patient should be registered whenever there is suspicion of a myocardial infarction or sudden coronary death. Every effort was

made to achieve as complete registration as was possible.

The study population consisted of the registered population of Helsinki under the age of 66. Those cases were also included where the attack occurred while the patient was not within the city limits.

The age limit was considered inevitable from the beginning for various reasons. Although aged persons only comprise ten per cent of the population of Helsinki, it could be estimated that extending the registration to include all age groups would have doubled the number of cases registered and the available personnel would have been unable to cope with the task. It is also well-known that even further difficulties in diagnosis of AIHD are encountered in the aged than in the young. It was also considered difficult to achieve an equally complete registration in the older age groups as in the younger ones, and it is also thought that the reliability of the information gained is less. Therefore, the registration was extended to all age groups merely over a short period.

The registration started on September 1st, 1969 as a pre-pilot study during which the propriety of the recording system was tested. After four months of this, the main study commenced at the beginning of 1970 without a break. The actual study period lasted for one year from January 1st to December 31st, 1970. The study period covering all age groups was from September 15th to December 31st, 1970. The second half of September was considered as a pre-pilot period and the incidence figures for older age groups are calculated from the three month period October–December 1970.

The personnel of the Ischaemic Heart Disease Register consisted of two, sometimes three interviewing nurses and one secretary with a physician in charge. The Register had an office in connection with the Finnish Heart Association's Research Unit.

This particular study is centred on one

important area in the field of the acute ischaemic heart disease, namely that of sudden death. However all the other cases found in the Register provide the requisite background, against which comparisons of various findings are made.

Sources of information

It was considered essential to the study to make sure that the highest possible percentage of relevant cases was indeed recorded. Therefore, close co-operation was sought with all persons, institutes and authorities from whom aid could be received to achieve this aim.

All hospitals in the city having wards for internal medicine were visited by the author and his coworkers. These hospitals are mentioned in the preceding chapter. The aim of the study was pointed to and promises of co-operation were exacted. In each hospital one of the physicians was appointed to be liaison. Special attention was paid to ensure collaboration of the personnel in the four outpatient departments of the hospitals with a 24-hour duty which are those through which most of the hospitalized patients passed. A circular letter addressed to the physicians and to the nurses in charge was delivered to all departments of internal medicine in every hospital. A further copy went to the filing staff of each hospital. The author paid visit in person to most of the wards, to all outpatient departments and to all files before or at the beginning of the pre-pilot study. Good co-operation was achieved in all but one hospital in this exceptional instance too satisfactory arrangements could be made for registration before the main study was undertaken.

Circulars, as well as articles in the local medical journals, were employed to make all physicians in Helsinki and in its environs aware of the study by which they were requested to report all patients under the age of 66 years whose case suggested possible

myocardial infarction or who met with sudden death. This could be done by post or telephone an automatic recorder after office hours being provided to make the reporting as convenient as possible.

The Alarm Centre dispatching doctors on duty on house calls was advised of the study. All physicians thus sent out by the Alarm Centre were requested to report the cases which they encountered. In addition, a special dispatching form was distributed to the physicians, requesting that it be used whenever a patient was sent to hospital under suspicion of a myocardial infarction. Since telephone calls received at the Alarm Centre are scrupulously clocked, reliable fixing of the time of a request for medical aid was possible.

All departments of pathology and forensic medicine in Helsinki performing autopsies were visited in person and informed of the study. A special report form was distributed to the pathologists with the request that it be completed whenever a fresh myocardial infarction was found or in case ischaemic heart disease was considered to be an essential cause of death. Most of the autopsies of sudden death cases occurring outside hospitals are made at the Department of Forensic Medicine, University of Helsinki, where a member of the register team was working at that time. He made regular checks of all autopsies performed at the department.

The Police gave a daily report on all medically unattended deaths outside hospitals of which they were notified. Finnish law prescribes that all such cases have to be reported to the Police. A police file is made of them, and the police are also authorized to order an autopsy.

The National Pensions Institute, which also administers the health insurance system of Finland, made available all those certificates submitted to it to substantiate daily allowance claims in which a diagnosis referring to ischaemic heart disease (ICD code

410—412) appeared. Copies of relevant certificates were sent to the Register regularly.

The City's ambulance service and the private ambulance companies were contacted. The clocking of the calls by the ambulance centre and the times when patients were reached were made available to the Register.

Full support was also given to the Register by the city authorities for public health.

Work procedure of the IHD Register

Abundant co-operation was promised at the beginning by all parties concerned. However it was soon found that the reporting carried out by the physicians was far from complete. There were also failures on the hospitals' side. It was therefore made standard practice adopted during the first weeks of the pre-pilot study that the nurses of the Register paid regular visits to the outpatient departments of the hospitals on a 24-hour duty basis and checked all diagnoses on admission made during the time between visits. Daily visits were paid to the largest hospital (University Central Hospital Meilahti) and the other hospitals were visited 2—3 times weekly. Furthermore, regular visits were made to all wards of these hospitals in order to check whether all cases had indeed been registered. Telephone inquiries constituted the means by which efforts were made to maintain co-operation with the other hospitals.

The death certificates were checked twice every month, and all relevant information was derived from them. The closest relative, if known, was then approached by letter requesting an interview and distinctly stating its purpose. If no relatives were known, the official population register was checked. If necessary the address was obtained from the Address Office. After approximately one week, one of the nurses made contact by telephone or in person. If no information was available the police reports concerning

those cases of which the police were notified were checked. If necessary the doctor who had signed the death certificate, or persons who had witnessed the death or any others with relevant information were contacted. A considerable number of those who died outside any hospital were recorded at the Department of Forensic Medicine before their death certificate was available.

The medical certificates substantiating claims for per diem sickness allowances of which copies were regularly received by the Register revealed some of those patients who had not been registered before. This was often the sole source of data causing inclusion in the register when the onset of illness had occurred outside Helsinki and the patient had survived. The patient was contacted at the earliest possible time after registration.

The staff of the Register carried out all the interviews, and the nurses also checked the information contained in hospital records. In each instance a special record form was completed. This procedure is common to all ischaemic heart disease registers incorporated in the programme of WHO although additions indicated by local requirements have been made. The procedure was followed through, no matter whether the patient was alive or dead after the interview any revisions and additions were made, and the time intervals involved were elicited from various sources. The interviews of hospitalized patients were mainly made at the hospital as soon as the patient's clinical status allowed. Most of the interviews of dead patients' relatives were made at their homes only relatively few were conducted at the Office of the Register. Some few interviews were made by telephone if the relatives declined to have an interviewer visit them. The intervening time between the death and the interview varied greatly. Most interviews were made within one month after death, but there were also longer intervals.

All registered cases were followed up for 4 weeks from onset of the acute attack, or until their death. After this period the interview record forms and all relevant information were gathered together: the author personally checked the interview records, hospitals records and autopsy reports and classified the diagnosis made of the acute attack according to the principles of WHO which are set forth later.

On conclusion of the study year all death certificates from this year which had accumulated in the Central Statistical Office of the State were checked once again. Six cases not registered before were then found. Furthermore all diagnoses on discharge from the University Central Hospital during the above-mentioned year were listed by computer: the same procedure was applied at the Statistical Office of the City of Helsinki concerning all city-owned hospitals. By means of this check, hospitals treating more than 90 per cent of all hospitalized myocardial infarction patients in Helsinki were covered. 48 cases not registered before were thus found.

Highly satisfactory co-operation was achieved both with patients and with the relatives of dead persons. Refusals for interview were very few. It is natural that it was more difficult to gain information concerning those who had died, only a police report was available in some instances. But the relatives of dead persons, when contacted, invariably displayed a fully positive attitude towards the study and were highly co-operative.

3. DEFINITIONS

Acute ischaemic heart disease (AIHD)

Acute ischaemic heart disease is defined as an episode fulfilling the criteria of WHO which are stated below for one of the three diagnostic categories: definite acute myocardial infarction, possible myocardial in-

farction, or insufficient data. An acute attack was considered to last up to 4 weeks. Any recurring attack, or death later than within four weeks, was considered a new event.

Sudden coronary death (SCD)

Sudden coronary death is defined as being an acute attack of ischaemic heart disease terminating fatally within one hour from the onset of the symptoms. The attack itself is required to fulfill the minimum criteria of AIHD given by WHO but it has to be realized that all sudden fatalities fulfill the criteria of the insufficient data category when no other diagnosis is obvious.

According to the definition, these deaths are witnessed, although there are a few exceptions. Some cases could be included in the group of sudden deaths notwithstanding the fact that the death was unwitnessed. Examples of this are those cases in which the victim had been seen in a normal condition and had been found dead soon afterwards. Cases in which a patient hospitalized for other causes died suddenly in hospital have been included in the SCD group.

Non-sudden coronary death (NSCD)

Non-sudden coronary death is defined as an acute attack terminating fatally within the first 28 days of the onset of symptoms, but not within the first hour. The attack is required to fulfill the minimum criteria of AIHD given by WHO. Unwitnessed deaths with unknown duration of the fatal attack were not included in this group either.

Unwitnessed death

Unwitnessed death is defined as a fatal attack of AIHD which fulfills the criteria of AIHD given by WHO but in which the terminal episode is not witnessed and the duration of the attack is therefore not known.

Survivors (SURV)

As survivors those patients with AIHD are considered who survived the first 28 days after onset of the acute attack.

Diagnostic categories

Four weeks after the onset of symptoms all registered cases were assigned to the different diagnostic categories on the basis of available information, and according to the criteria given by WHO at that time (Working Group of Ischaemic Heart Disease Registers, EURO 5010 (4), 1970). Minor revisions by WHO have later transpired concerning assignment to the categories Possible myocardial infarction or Insufficient data whereas the minimum criteria of acute ischaemic heart disease and the criteria for definite myocardial infarction have remained unchanged.

The registered episode was diagnosed on the basis of four criteria: history of the acute attack, electrocardiograms, serum enzyme levels, and post mortem examination. Quite naturally information concerning all four factors was not always available. In many instances of sudden death there was nothing but the result of post mortem examination, if any. Those cases are also numerous in which a history of acute chest pain was recorded while electrocardiogram and serum enzyme levels were usually absent. The four diagnostic categories and their definitions are listed below.

1. Definite acute myocardial infarction

- Unequivocal ECG evidence of recent myocardial infarction, =
- Equivocal ECG evidence of recent myocardial infarction with elevated levels of appropriate serum enzymes, or
- Elevated levels of appropriate serum enzymes with a typical history of chest pain, or
- Positive post-mortem evidence

2. Possible acute myocardial infarction.

- A typical history of pain without evidence justifying assignment of the case to the first category

3. Insufficient data.

- Insufficient items of evidence justifying assignment to the above two categories, while no diagnosis other than that of ischaemic heart disease has been made. Most cases of sudden death belong to this category

4. No myocardial infarction.

- Cases in which another diagnosis was made or which did not fulfil the criteria for assignment to any one of the above-mentioned three categories. These cases were excluded from the study

The definitions for unequivocal and equivocal ECG changes have been given by WHO earlier (Hypertension and coronary heart disease, 1959 and Working Group of Ischaemic Heart Disease Registers, EURO 5010 (4), 1970).

The following definition for elevated serum enzyme levels was applied in this study: SGOT 30 IU/ml or more, or SGOT 20–29 IU/ml and LDH was over 240 and isoenzymes 1 and 2 were elevated. The LDH isoenzymes were, however determined in exceptional cases only.

The definition for the typical history of chest pain is defined by WHO earlier (Arterial hypertension and ischaemic heart disease, 1962).

The definition for positive post-mortem evidence of an acute myocardial infarction in this study according to WHO is: The presence of a fresh myocardial infarction and/or the presence of a recent thrombotic occlusion of a coronary artery observable by the naked eye. Only the naked eye appearance counted as positive evidence.

Occlusion, stenosis or calcification of the coronary arteries, scars after old infarctions, fibrosis etc. were also recorded. It is clear

that they furnished valuable aid in the determination of the cause of death, even though actual acute changes were absent.

Age

The age at onset of symptoms.

Marital status

The subjects are divided into married, single divorced and widowed persons.

Social class

The division into four social classes is based on the subjects' occupation. The same division is used in the census in Finland.

Class I. Comprises professionals, higher officials, managers etc.

Class II Consists of employers in small enterprises, the self-employed, technicians, foremen, higher clerical workers, nurses etc.

Class III Consists of skilled workers, lower grade clerical employees, shop assistants, drivers, and workers on a similar level etc.

Class IV Consists of various kinds of unskilled workers, domestic helps, char women, porters etc.

The retired persons were classified according to their previous occupation. The final analysis of the association of acute ischaemic heart disease and social class was made both including and excluding the retired persons.

Housewives are classified according to their own occupation if possible. Those with no occupation of their own are referred to the social class of their husbands and widows to that of their late husband.

Premonitory symptoms

The patients and their relatives were questioned as to premonitory symptoms during 28 days preceding the onset of the attack. The following symptoms were particularly inquired after the fresh occurrence of

angina pectoris or the exacerbation of existing angina pectoris discomfort in the chest, heaviness of the arms, nausea, unusual tiredness, unusual dyspnoea and the first occurrence of palpitations. Other specific symptoms reported to the interviewer were also recorded. Symptoms with onset earlier than 4 weeks before the attack were not recorded unless distinct exacerbation of the symptom had been noted during the preceding month. Visits to a doctor within 12 or 4 weeks before onset of the attack were recorded together with the cause of each visit. Of several visits made during the preceding month only the last one was recorded.

Previous diseases

The information on previous diseases was gained by the interviewers from the patients or their relatives, and from hospital records. Whenever possible the hospital case records of earlier hospitalizations were also checked. According to the recommendation by WHO any history of myocardial infarction, hypertension, stroke or diabetes mellitus was divided into two validity categories: confirmed if there were hospital case records or other medical documents confirming the patient's history and non-confirmed if no such confirmation could be found but if the patient's history was consistent with the diagnosis. In the presentation of results these two validity categories were combined.

Myocardial infarction

A confirmed myocardial infarction included in the patient's history was required to fulfill the diagnostic criteria laid down in this study for a definite myocardial infarction. If confirmation was not possible even though the history was consistent with the diagnosis, a non-confirmed episode was recorded. In those cases where more than one myocardial infarction had been reported, only the confirmed episodes were taken into account.

Angina Pectoris

The previous definition of WHO (Arterial hypertension and ischaemic heart disease 1962) for effort angina constituted the basis. A history of chest pain was accepted as positive if a physician's opinion stated that angina pectoris was concerned. If it appeared to the author that the documented description of chest pain contained in the interview record form was compatible with typical effort angina, this was also accepted as a positive symptom.

Hypertension

If diagnosis of hypertension had previously been made by a physician, the history was accepted as positive, either confirmed or non-confirmed, depending on the availability of confirming documents. Hypertension detected after admission owing to the registered episode was not included.

Other cardiovascular diseases

This category consists of diseases such as a stroke, valvular heart disease, pulmonary heart disease and chronic disturbance of the cardiac rhythm. Congestive heart failure was also included. The group constitutes, therefore, a heterogeneous assembly of diseases, part of which no doubt represent symptoms of ischaemic heart disease or of other cardiovascular diseases.

Diabetes mellitus

The diagnosis of diabetes mellitus was accepted as confirmed if it was confirmed by previous medical documents. Constant antidiabetic treatment was also accepted as positive proof. If the patient's history was unsupported, it was considered as being non-confirmed.

Obesity

The height and weight of the patients were obtained from medical records in the hospitals or by inquiry from the patients or their relatives. In some cases only measurements made in connection with autopsy were available.

As an index of relative weight a body mass index, that is Quetelet's index, weight/height-squared was used.

Smoking habit

The patients were first divided into three categories: current smokers, ex-smokers and non-smokers.

The definitions for the different categories are

A smoker is one who smokes at least one cigarette per day (or an equivalent quantity of pipe or cigar tobacco) or who has smoked at this level up to a time later than three months before the acute attack.

An ex-smoker is one who has smoked at the level defined above but has given up smoking not later than three months before the acute episode.

A non-smoker is one who has never smoked tobacco at the levels indicated above.

The cigarette smokers were further divided according to the number of cigarettes consumed daily while the pipe and cigar smokers constituted a category of their own. Those smoking both cigarettes and pipe or cigars were included in the group of cigarette smokers.

Physical activity

A particular questionnaire was completed for every patient concerning their daily activity on the job, the time spent walking, sitting or standing, their means of getting to and from work, the frequency of lifting or carrying heavy objects, etc. A similar set of questions was asked concerning the pa-

tients' off the-job activities, the indulging in sport, walking gardening and household work.

On the basis of the questionnaires, the author assigned the patients to three activity categories with regard to the on-the-job and off the-job activities — both separately

On the job activity

Class I Inactive group. Mainly sitting. Never or very rarely lifting or carrying heavy objects.

Class II Medium group. Work also comprising walking. Occasional lifting or carrying of heavy objects.

Class III Active group. More walking than sitting. Frequent carrying or lifting of heavy objects. Tasks causing breathlessness or sweating are frequent.

Off the job activity

Class I Inactive group. Virtually no physical activity during leisure time.

Class II Medium group. Walking cycling or moderate physical work at home at least 4 hours weekly

Class III Active group. Sports or heavy work at home regularly at least 4 hours weekly

An attempt was made to test the validity of the questionnaire by interviewing a group

of 87 coronary heart disease patients of the same age who did not belong to the study material. In addition to the questionnaire employed in this study a recall record form was used and the mean calory expenditure was calculated for each one of these patients. The author assigned them to the three activity groups on the basis of the same principles which were applied to the patients of the actual study

Table 2 presents the results of this test. It is seen that most of the cases are concentrated in the inactive categories. The differences between various categories are statistically only almost significant. On the other hand, it is acknowledged that the validity of the method of comparison is questionable.

Place of onset of symptoms and of death

The place of onset of symptoms was classified as follows at work, at home, in a hospital, or in another place. In some cases there was no definite onset of symptoms and it was also naturally impossible to define the place of onset.

The place of death was classified in the same manner except that those who died during transportation to a hospital or at a hospital's outpatient department within one hour of arrival were referred to extra groups of their own.

Table 1. Mean daily calory expenditure of 87 patients with IRD in different job-connected and leisure time-connected physical activity categories (numbers of patients in parenthesis).

Physical activity connected with leisure time	Job-connected physical activity			All cases
	I	II	III	
I	2485 (10)	2543 (7)	402 (1)	2503 (18)
II	2322 (40)	2588 (4)	3596 (3)	2529 (47)
III	2639 (16)	2624 (6)	2323 (1)	2713 (23)
All cases	2544 (66)	2642 (16)	2968 (5)	

$$F = \frac{s_p^2}{s} = 1.25$$

Activity at onset of symptoms

The subjects were considered to be at rest when sleeping, lying or sitting without engaging in any activity. The category at work comprises a wide variability of activities involving light or moderate physical stress. The exceptional cases of strenuous activity were separately coded.

4 MATHEMATICAL TREATMENT

Of the various quantitative variables measured in the study the means were calculated in the different groups (SCD, NSCD SURV) separately for both sexes and, as indicated, in different age groups, and their standard deviations were determined. Statistically significant differences between such means were established by Student's *t*-test. A one-way analysis of variance was applied in testing the mean calory expenditure in different classes of physical activity and with regard to body mass index. The comparison of proportions in different classes was carried out by means of the usual test based on an approximation of the normal distribution.

Distributions of various aspects in the different groups (SCD NSCD SURV) were displayed in contingency tables of a suitable size. Statistically significant differences between the groups (SCD NSCD SURV) were established by the goodness of fit test, based on an approximation of the chi-square distribution (e.g. Vasama and Varti 1972).

The original data concerning marital status, social class and physical activity were age-adjusted by a direct method of standardization (Armitage 1971). After standardization, the above-mentioned chi-square goodness of fit test was used in testing the statistical significance of the observed frequency distributions.

The statistical significance of differences is indicated by the following symbols

N.S.D	No statistically significant difference ($p \geq 0.05$)
	Almost significant difference ($p < 0.05$)
**	Significant difference ($p < 0.01$)
***	Highly significant difference ($p < 0.001$)

IV RESULTS

1 REGISTERED CASES OF ACUTE ISCHAEMIC HEART DISEASE — AGE, SEX AND DIAGNOSTIC CATEGORIES

All cases of acute ischaemic heart disease in those aged 35 years or younger registered within the population of Helsinki, constitute the patients in the present study. Some of their basic characteristics are described in the following

Results

Altogether 1267 cases fulfilling the minimum criteria of WHO for AIHD were registered. 953 (75.2 per cent) were males and 314 (24.8 per cent) were females. Table 3 and Fig. 2 present the patients by age, sex and diag

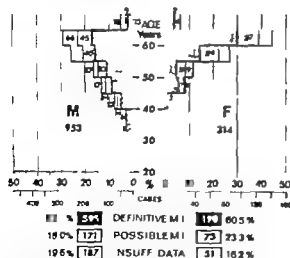


Fig. 2. Age and sex distributions of acute ischaemic heart disease cases in different diagnostic categories of WHO: numbers of cases and percentages of male and female cases in each category

nostic category of WHO as defined on page 25. The number of cases in both sexes increases rapidly with age. The mean age of females was higher (57.9 years) than that of males (54.9 years). The different age distribution between both sexes is clearly evident from Fig. 2, the majority of female cases being concentrated in the oldest age groups.

Table 3. Distribution of acute ischaemic heart disease cases by age, sex and WHO diagnostic category

Age	Males			Total
	Definite	Possible	Insuff data	
—29	1	—	—	1
30—34	7	—	—	7
35—39	12	10	6	28
40—44	48	14	12	74
45—49	88	24	17	129
50—54	104	33	17	154
55—59	162	40	31	233
60—64	163	45	66	274
65	23	8	18	49
Total	895	171	187	1253
Per cent	62.4	18.0	19.6	100.0

Age	Females			Total
	Definite	Possible	Insuff data	
—29	2	—	—	2
30—34	1	—	—	1
35—39	—	1	1	2
40—44	1	1	2	4
45—49	18	7	1	26
50—54	16	11	9	36
55—59	46	24	14	84
60—64	85	27	18	130
65	11	2	6	19
Total	190	73	51	314
Per cent	60.5	23.3	16.2	100.0

Table 3 and Fig. 2 also reveal the relative proportions of the different WHO categories in both sexes. 82.4 per cent of male and 60.5 per cent of female cases fulfilled the criteria of definite myocardial infarction. The relative proportion of all WHO categories is roughly the same in different age groups.

Table 4 shows the age and sex composition of the study group of 239 sudden coronary death cases (SCD) in juxtaposition with the two comparison groups, that of 240 non-sudden coronary death cases (NSCD) and that of 736 survivors (SURV). The small group of 52 unwitnessed deaths is also presented. The proportion of dead cases from all AIHD cases in different age groups is presented in Fig. 3 which indicates also the case fatality rate, 43.6 per cent for males and 37.1 per cent for females. In Fig. 4, the age and sex composition of all fatal cases of AIHD is presented in three subgroups: sudden deaths, non-sudden deaths and unwitnessed deaths.

It is noted that SCD cases show an even greater male prominence (83.7 per cent) than all AIHD cases (75.1 per cent); this is especially noteworthy in the youngest age groups. There are no female sudden death cases under the age of 40 years, in contrast to six male cases, and only one female case occurs in the next age group, from 40 to 44 years, as opposed to ten males.

In each one of the comparison groups the mean age for males is lower than that for females. The males' mean age is closely equal in all groups consisting of deceased persons, 56.1 years in the NSCD group and in the group of unwitnessed deaths and 56.2 years in the SCD group. It is definitely lower in the SURV group (53.9). The females' mean age is highest in the group of unwitnessed deaths, 59.5 years, followed by the NSCD group, 59.2 years. The mean age is lower in the SURV group (57.5) and lowest in the SCD group (57.2 years). If both sexes are combined, it can be found that the mean age is definitely lowest in the SURV group

(54.9 years), followed by the SCD group (56.2 years). The mean age in the group of unwitnessed deaths is even higher (57.0 years).

Sudden death constituted 18.6 per cent of all registered cases of AIHD, 21.0 per cent of male and 12.4 per cent of female cases. The male:female ratio of all cases of AIHD was 2:1; that of all fatal cases of AIHD was 4:1, but in the SCD group it had the ratio 5:1.



Fig. 3 Age and sex distributions of acute ischaemic heart disease cases, among survivors and fatal cases: numbers of cases and percentages of male and female fatal cases.

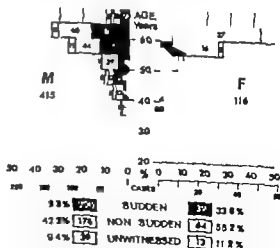


Fig. 4 Age and sex distributions of fatal acute ischaemic heart disease cases in SCD, NSCD and unwitnessed death groups: numbers of cases and percentages of male and female cases in each group.

Comments

Considering the number of registered cases of AIHD it must be understood that only persons in the working age group are concerned. The estimated number of AIHD in the age groups older than 65 calculated from the three-month study period, consisted of 1312 cases. This means that the persons of working age forming the object of this study only constitute half of the total burden of AIHD borne by the health services and by the medical care system of the community.

The percentage of the registered cases per month increased approximately by ten from the pre-pilot study period, probably owing to more complete coverage after the registration system had become firmly established. The checks on the perfection of the registration disclosed a total of 54 cases which had escaped notice. This is 4.5 per cent of all registered cases. It is likely that those who die in AIHD are more positively registered than any others considering that the law prescribes that any death certificates have to be sent to the city authorities independent of the residents place of death, and that all death certificates are gathered at one point. The registration of survivors has probably not been equally complete. The checking process probably covered the majority though not all, of the cases hospitalized in Helsinki. The cases treated outside Helsinki were registered if prescribed sick leave and if they were not already receiving a disability pension on the basis of substantiating claims for a per diem allowance. Only a few surviving patients with AIHD entered the Register from other sources, reported, for instance, by their attending doctor. Therefore it must be considered that a certain number of cases has escaped notice. In Finland, nearly all patients in whom myocardial infarction is even suspected are sent to a hospital. Almost the only exception consists of cases which have eluded diagnosis before the acute phase has

Table 4. Age and sex distribution of acute ischaemic heart disease cases in different groups.

Age	Sudden deaths		Non-sudden deaths		Unwitnessed deaths		Survivors		All AIHD cases	
	M	F	M	F	M	F	M	F	M	F
—29	—	—	—	—	—	—	1	2	1	2
30-34	2	2	—	—	—	—	5	1	7	1
35-39	4	4	6	1	—	—	13	1	23	2
40-44	10	1	13	—	5	—	46	2	74	4
45-49	25	3	31	3	8	—	83	20	126	28
50-54	20	6	28	3	1	2	104	25	134	38
55-59	53	13	66	16	11	2	133	53	243	84
60-64	67	13	80	37	11	7	136	52	274	140
65	16	3	13	4	5	1	11	11	46	19
Total	200	39	239	72.6	39	13	538	192	953	314
Per cent	83.7	16.3	77.0	25.0	73.1	25.0	73.1	24.9	75.1	24.9

Table 8. Distribution of acute ischaemic heart disease cases in different groups by age, sex and WHO diagnostic category.

Age	Sudden deaths			Non-sudden deaths			Unwitnessed deaths			Survivors			All AHD cases		
	Def.	Poss.	Tot.	Def.	Poss.	Tot.	Def.	Poss.	Tot.	Def.	Poss.	Tot.	Def.	Poss.	Tot.
	data			data			data			data			data		
Males															
20-24	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—
25-29	2	—	2	—	—	—	—	—	—	3	—	—	3	—	—
30-34	1	—	1	4	2	6	—	—	—	7	8	15	12	10	22
35-39	5	3	8	5	1	6	3	—	3	36	10	—	46	14	60
40-44	13	5	18	7	2	9	1	—	1	68	17	—	85	24	109
45-49	6	3	9	21	3	24	—	—	—	77	37	—	104	33	137
50-54	12	8	20	35	4	39	1	—	1	104	30	—	135	40	175
55-59	12	6	18	29	10	39	2	—	2	110	26	—	136	45	181
60-64	4	2	6	7	—	7	3	—	3	10	3	—	13	5	18
65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	54	28	82	118	22	140	8	—	8	415	121	2	536	171	707
Per cent	27	14	41	57	12	59	21	0	78	77	22	1	85	18	100
Female															
20-24	—	—	—	—	—	—	—	—	—	2	—	—	2	—	—
25-29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30-34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
35-39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
40-44	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—
45-49	2	1	3	3	—	3	—	—	—	13	6	—	19	2	25
50-54	4	1	5	2	—	2	—	—	—	14	11	—	25	7	36
55-59	3	2	5	8	3	11	1	—	1	23	20	—	43	11	54
60-64	—	—	—	28	4	32	2	—	2	62	21	—	83	27	110
65	—	—	—	2	—	2	—	—	—	9	2	—	11	2	13
Total	9	4	13	43	7	50	3	—	3	134	62	1	196	73	269
Per cent	23	10	33	86	11	97	22	0	78	85	32	1	93	23	100

passed. Of these cases, too reports should be received by way of the certificates for substantiating per diem claims, with the same reservation as has been mentioned above.

To summarize the foregoing, it can be said that obviously the registration of fatal cases has been fairly complete, while that of survivors has been less perfect. Therefore the mortality figures may be slightly exaggerated. The checking procedure which was undertaken justifies the assumption that this over-estimation cannot be of any substantial order. It is natural that cases with unrecognized myocardial infarctions are never discovered in a study of this kind. According to several prospective epidemiological studies, the number of such cases seems fairly substantial (Rosenman et al. 1967 a, Master and Geller 1969 Kannel et al. 1970).

The definitions of the different groups, i.e., sudden coronary deaths (SCD), non-sudden coronary deaths (NSCD) and survivors (SURV), and that of unwitnessed deaths have been given earlier (pages 24—25). The group of unwitnessed deaths posed a special problem. It is likely that they represent, in part, sudden deaths and non-sudden deaths, in part, but the relative proportions of both are not known. Therefore, it was not considered proper to include them in either one of the above-mentioned groups. Their number is comparatively small, only 52 cases, i.e., 4.1 per cent of all AIHD cases. In the subsequent analyses, this group is included in the total of AIHD cases and it is treated as a separate group, while the results referring to it are not stated in all instances.

The results show that AIHD is more common among males than among females. The attack afflicts males at younger ages than it does females. The definitely lower mean age of the survivors, compared with those who die during the attack, can be interpreted to mean that age is a factor increasing the mortality rate.

Table 5 shows that according to the criteria

of WHO the validity of the diagnosis of AIHD can be considered best in the SURV group, followed by the NSCD group. It is better in both groups than in the SCD group. This is hardly unexpected and depends on different possibilities as regards confirming the diagnosis in the different groups. The basis of the diagnosis in the SCD group will be discussed in detail in Chapter IV.3

Summary

1267 cases of AIHD in persons aged 65 years or younger were registered during the study year. However this is only half of all cases provided all ages are considered. 239 cases belonged to the SCD group, 240 to the NSCD group, 52 cases to the group of unwitnessed deaths and 736 cases to the SURV group. The case fatality rate was 43.6 per cent for males and 37.1 per cent for females. Male prominence was obvious in all comparison groups. It was most accentuated in the SCD group. The mean age of females was 57.9 years and that of males 54.9 years. 62.4 per cent of male and 60.5 per cent of female cases fulfilled the criteria of definite myocardial infarction. Sudden death constituted 18.8 per cent of all registered cases of AIHD, 21.0 per cent of male and 12.4 per cent of female cases.

2. RELATIVE PROPORTION OF SUDDEN DEATHS FROM ALL ACUTE ISCHAEMIC HEART DISEASE DEATHS AND INCIDENCE OF SUDDEN CORONARY DEATH

In considering the frequency of sudden coronary death in a community two different approaches are possible. On one hand the mortality of AIHD may be considered as a function of time and the relative number of sudden deaths, with reference to all AIHD deaths or to all AIHD cases,

can be determined. On the other hand, one may calculate the incidence of sudden coronary death. Both procedures enable the results to be compared with results from other comparable studies. In the following an attempt has been made to apply both manners of evaluating the frequency of SCD in the present study

Results

In altogether 478 fatal cases of AIHD in 378 male and 103 female cases, the time interval from the onset of symptoms to death was known. Fig 5 showing the cumulative mortality of both sexes as a function of time reveals a high acute mortality in AIHD attacks. At the cut point of this study at one hour after the onset of symptoms, the different course of AIHD attack of males and of females is clearly evident. 53.1 per cent of the males who died during the first four weeks after the attack, as opposed to only 38.5 per cent of such females, died within the first hour. If only those cases are considered in which death occurs within 24 hours, the critical importance of the first hour is also very clearly discernible (Fig 6)

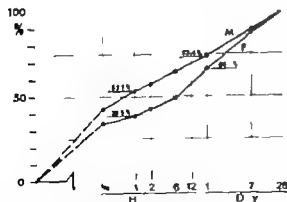


Fig 5 Cumulative mortality graphs of male and female patients with acute ischaemic heart disease who died within 28 days, according to interval from onset of attack to death.

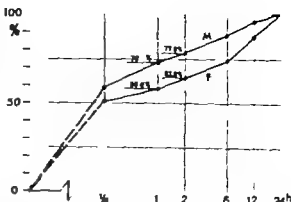


Fig. 6 Cumulative mortality graphs of male and female patients with acute ischaemic heart disease who died within 24 hours, according to interval from onset of attack to death.

72.4 per cent of these males and 58.0 per cent of these females were dead after the first hour had passed. Figures 5 and 6 also reveal that most of the deaths occurring within one hour are in actual fact momentaneous, or at least terminate within 15 minutes.

If those cases are also taken into account where the exact time interval from the onset of symptoms is not known, there were altogether 531 deaths in AIHD within four weeks: 415 (78.2 per cent) of them males and 116 (21.8 per cent) females. Sudden death constituted 44.9 per cent of these deaths (48.2 per cent of the males and 32.6 per cent of the females)

Fig. 7 shows the incidence of sudden coronary death as well as that of AIHD for both sexes as a function of age. It can be seen that all four incidence graphs (presented in logarithmic ordinates) constantly ascend with age. Both female incidence curves run throughout on a lower level than the corresponding male curves. The sex difference is more prominent in the case of SCD. While the female incidence of AIHD reaches the males' level with a delay of roughly ten years, the corresponding time interval is closer to 15 years for the sudden death graphs.

10000

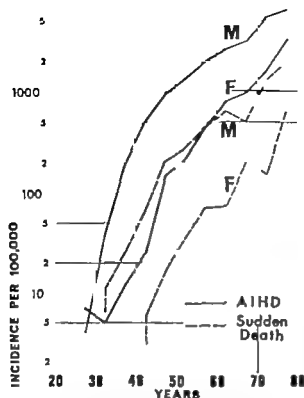


Fig. 7 Age-dependent incidence of acute ischaemic heart disease and of sudden coronary death in males and females, per 100 000 inhabitants of Helsinki. Parts of the graphs (age groups over 65 years) based on three month study period distinguished by thinner lines.

Comments

Several community and prospective studies have consistently pointed out the critical significance of the first hours in an attack of acute ischaemic heart disease. Within one hour 38 to 75 per cent of all AIHD deaths occur.

In the Edinburgh Community Study death within one hour constituted 47.8 per cent of fatal male and 38.9 per cent of fatal female cases (Fulton et al. 1969). In Belfast, the corresponding figures were 40.7 per cent for males and 40.4 per cent for females, calculated from all IHD deaths within 4 weeks with known duration of the last attack (McNeilly and Pemberton 1968). In Framing-

ham, 57.8 per cent of male and 38.9 per cent of female fatal cases terminated within one hour (Gordon and Kannel 1971), and in Tecumseh 45.9 per cent of fatal cases of both sexes respectively (Chiang et al. 1970). In the East West Study a prospective cardiovascular study carried out in two parts of Finland, 51.1 per cent of IHD deaths occurred within one hour (Punsar 1971). Several other studies have reported similar results (Eisenberg et al. 1961, Spiekerman et al. 1962, Bainton and Peterson 1963, Mathewson et al. 1965, Kuller et al. 1966 a, Fry 1968, McWhinney 1968, Stamler et al. 1969).

Compared to the studies cited, the results in the present study seem to be fairly similar. A distinct male preponderance is seen in this study in agreement with most of the previous studies mentioned above.

Another aspect with reference to sudden coronary death is illustrated by the incidence figures. Such figures previously reported are few and they are based on studies in which widely different definitions of sudden coronary death have been applied. Results from certain studies are compiled in Table 6. Only the Framingham and Tecumseh studies employed a time interval of one hour. From the Edinburgh Community Study the relative proportion of deaths occurring within one hour was reported earlier (Fulton et al. 1969), but incidence figures are only available concerning medically unattended deaths (Armstrong et al. 1972). The data from Stockholm likewise concern medically unattended deaths, deaths in hospitals or in other institutions not being included (Wiklund 1971). In Baltimore, a time interval of two hours was used (Kuller et al. 1967), while in the HLP study New York, no definite time interval was stated (Weinblatt et al. 1968).

Table 6 reveals that the male incidence discovered in the present study is very close to that in Framingham and in Edinburgh. It is obviously higher than in Baltimore, New York and Stockholm. Comparison with the incidence in the Tecumseh Study (Chiang

Table 6. Age-dependent incidence of sudden coronary death in different studies, per 1000 inhabitants per year

Age	Males						
	Heisinki	Framingham	Baltimore	HLP New York	Stockholm	Edinburgh	Tecumseh
30-34	0.1						M and F
35-39	0.3	0.5			0.1		0.3
40-44	0.7				0.3		
45-49	2.1	1.5			0.6	1.3	1.0
50-54	1.7		2.0	1.4	0.7		
55-59	4.5	2.5			2.0	3.2	2.0
60-64	8.8			1.5	3.5		
65-69	5.0				5.0	5.7	5.5
70-74	13.1				7.7		
75-79	19.5				13.5		8.7

Females							See above
40-44	0.1						
45-49	0.2	0.2	2.0	0.2	0.1		
50-54	0.4				0.2		
55-59	0.7	0.5			0.3	1.4	
60-64	0.7			0.5	0.6		
65-69	2.0				1.5	2.5	
70-74	1.5				2.5		
75-79	7.1				4.1		

et al. 1970) is difficult since the figures stated are concerned with both sexes however the incidence is probably higher in Tecumseh. The incidences given for females are very close only those of Edinburgh are distinctly higher. Sudden coronary death is relatively rare among females and reaches a level comparable with the males in age groups which are 10 to 15 years older. To summarize the foregoing, it can be stated that the present study furnished results that are on the same lines as previous studies, reported from countries such as the U.S.A. and England, where incidence of IHD in general is also high.

When the frequency of SCD is considered it should be realized, however that even

though the time limit is accurately defined, the reported duration is merely a rough estimate made by persons witnessing the fatal attack. Under these circumstances, it is certainly inadmissible to assume that the relatives would be capable of a reliable determination of the duration of the fatal attack. This biasing effect is offset, to some extent, by the fact that the majority of deaths occurring within one hour are in fact momentaneous, thus definitely ranging with in the time limit given. On the other hand, in all likelihood some of the persons apparently succumbing to sudden death have actually had definite symptoms more than one hour prior to death, which they have not disclosed to their relatives.

Therefore, it appears that in spite of an accurate definition of SCD the recorded number of SCD cases is only an estimate even at its best. Owing to the nature of the problem in itself exact information is impossible to obtain. These circumstances probably encumber studies in other areas as well. The sole means by which reasonably comparable results may be achieved is to apply strictly uniform criteria and to make the study design also comparable in other respects. This will be achieved in the near future, as soon as results are available from all Ischaemic Heart Disease Registers comprised in the WHO comparative study.

Summary

The suddenness of death seems to be a typical feature in fatal AIHD cases. No less than 53.1 per cent of male, as opposed to only 38.5 per cent of female fatal attacks with known duration terminated within one hour. This remarkable sex difference is also reported earlier. The relative proportion of SCD from all AIHD deaths as well as the incidence of SCD both seem to be high, although figures on the same level have been reported from certain other countries. The possibilities of reliable comparisons are restricted by substantial differences between the definitions employed.

3 BASIS FOR DIAGNOSIS IN CASES OF SUDDEN CORONARY DEATH

According to the rules of WHO given above the diagnosis of AIHD is based on four criteria: a history of chest pain, a post mortem examination, electrocardiographic changes, and serum enzyme tests. It is natural that usually only the first two items, if any are available when the problem is to determine the cause of sudden death.

Autopsy is, no doubt the best support of the diagnosis, regardless of the fact that myocardial necrosis in experimental studies

only becomes visible after some 6 to 12 hours, and that the pathological criteria for diagnosis are anything but firmly established.

A history of acute chest pain is obtained in a certain proportion of SCD cases, but remarkably often the attack is momentaneous and according to reports without symptoms.

In addition to the above-mentioned criteria, a history of previous, diagnosed ischaemic heart disease, either myocardial infarction or angina pectoris, no doubt provides strong evidence for AIHD as being the cause of sudden death. The same concerns to a certain degree prodromal symptoms during the month preceding the fatal attack, especially a recent onset or the progressive character of previous angina pectoris.

The basis employed in the present study in determining the cause of death is discussed in the following, separately for autopsied and for non-autopsied cases. Case histories are presented in those cases in which the diagnosis is considered to have remained uncertain.

Autopsied cases

In all, 192 of the total of 239 SCD cases (80.3 per cent) were autopsied, namely 159 out of 200 males (79.5 per cent) and 33 out of 39 females (84.6 per cent).

Conventional autopsy technique was applied at most autopsy places. In addition to macroscopic examination of the myocardium the coronary arteries were longitudinally dissected, a search was made for evidence of a fresh thrombus and the degree of stenosis was estimated. Microscopic in addition to macroscopic examination was very frequent. It was performed in 119 cases in all (62 per cent of the autopsied SCD cases). At the department of forensic medicine at the university where the majority of SCD cases was autopsied (78.6 per cent of all autopsied SCD cases) a more detailed method for examination of the heart pro-

posed by WHO (Working group on Ischaemic heart disease registers, EURO 5010, 1969) was used during the study period. The method included e.g. histochemical staining by employing a dehydrogenase reaction. The results of the staining were not, however used as a basis for diagnosis but only for the selection of histological specimens.

The autopsied cases were divided into three main categories according to the autopsy finding

1. Cases with recent myocardial infarction and/or a fresh thrombus in the coronary arteries established either in macroscopic or in microscopic examination. It is noted that only naked eye appearance is accepted as positive post mortem evidence when the cases are allocated to WHO diagnostic categories.

2. Cases with acute ischaemic changes in the heart muscle, not classifiable as acute myocardial infarction.

3. Cases showing no acute changes in the myocardium.

Scars after old myocardial infarctions and the degree of coronary arteriosclerosis were also recorded and they were considered to provide valuable support for the AIHD diagnosis in the two latter categories.

Coronary arteriosclerosis was defined as being severe provided it had progressed to total occlusion in at least one of the main coronary arteries. The grade was recorded as moderate if at least fifty per cent of the lumen was occluded and as slight if less occlusion had been discovered. Coronary arteriosclerosis was by no means absent, in these cases either

Table 7 presents the autopsy findings in the 192 autopsied SCD cases. The cases have been classified in the three main categories mentioned above and presented in a decreasing sequence according to the degree in which the autopsy finding supported the diagnosis of AIHD

It can be seen that in about 40 per cent of male and 30 per cent of female SCD

Tabl 7 Autopsied cases of sudden coronary death. Post mortem findings.

	Recent myocardial infarct or fresh thrombosis		Acute ischaemic changes		No	acute myocardial changes		
	Macroscopic diagnosis	Microscopic diagnosis	With old infarct	Without old infarct	With old infarct	Severe coronary arterio- sclerosis	Moderate coronary arterio- sclerosis	Slight coronary arterio- sclerosis
Males								
—49	20	2	3	4	6	2	1	2
50—59	16	8	12	2	20	2	2	1
60—65	18	6	13	6	13	—	6	—
Total	52	12	28	12	29	4	9	3
Per cent	22.7	7.5	17.6	7.5	24.8	2.8	8.6	1.9
	40.2		25.1			34.7		
Females								
—49	2	—	—	1	—	—	—	1
50—59	4	1	1	5	2	—	2	1
60—65	3	—	3	—	4	—	1	1
Total	9	1	4	6	6	—	4	3
Per cent	27.3	3.0	12.1	16.2	16.2	—	12.1	8.1
	30.3		30.3			39.4		

cases, autopsy indicated an evidence of a fresh myocardial infarct, or a fresh thrombus in the coronary arteries. In 11 male and one female case this was only revealed by microscopic examination and these cases could not be allocated to the WHO category >definite myocardial infarction. Nevertheless, the cause of death must be considered as confirmed in them, too.

Another 25 per cent of male and 30 per cent of female cases were classified in the second category showing acute ischaemic changes, such as soft consistency oedema, pallor etc. In about two-thirds of these cases myocardial scars furnished additional support for the AIHD diagnosis. However 12 male and 6 female cases presented only changes of acute ischaemia without any scars of an old infarct. Coronary arteriosclerosis was estimated to be severe in 5, moderate in 8 and slight in 5 cases. If severe coronary arteriosclerosis is considered as justifying the diagnosis of AIHD there remain 13 cases in which support for the diagnosis had to be sought from the case histories.

In 3 cases, typical chest pain was reported in connection with the fatal attack. The diagnosis of IHD was made during life in 8 cases and that of hypertension, in another 3 cases. In two cases a fresh occurrence of angina pectoris during the month preceding death was reported.

There remained only 4 cases, two males and two females, in which the coronary arteriosclerosis was of moderate or slight degree and the history did not lend any additional support for the diagnosis of AIHD. In them, an autopsy finding of acute ischaemia remained the only basis for diagnosis.

More than one-third of all autopsied SCD cases fell into the third category in which no acute changes in the myocardium could be found. In a notable percentage of these however strong evidence in favour of IHD

was provided either by means of myocardial scars or by means of advanced coronary arteriosclerosis.

If scars after an old myocardial infarct and severe coronary arteriosclerosis are considered, even in absence of any visible acute changes, sufficient evidence of AIHD as being a cause of death, 92.3 per cent of autopsied male and 78.8 per cent of autopsied female sudden death cases met up with this requirement. There remained, however 10 cases, 13 male and 7 female cases, in which the sole autopsy finding was coronary arteriosclerosis of moderate or lower grade. The grade of coronary artery stenosis was assessed as being moderate in 13 and as slight in 6 of these. It seems that in these 19 cases, again, additional support for the determined cause of death is called for.

A severe chest pain in connection with a fatal attack was reported in three cases. A history of previous myocardial infarction was obtained in one male and in one female case. In another seven male cases a history of angina pectoris during the patient's life was recorded. It may further be mentioned that a history of hypertension was found in four cases while a history of diabetes mellitus was found in none. In three cases a changing pattern of angina pectoris during the preceding month prior to death was reported.

After checking the case histories in those 19 cases mentioned above, there still remained 6 cases (11 males and 4 females) in which neither the autopsy nor the patient's history could be considered to furnish sufficient evidence for diagnosis of AIHD. Coronary arteriosclerosis was assessed to be moderate in three and slight in the other three cases. Although other major causes of death were excluded, the cause of death without doubt remained uncertain.

The case histories of the above-mentioned six cases were as follows

1. A 48 year old male who succumbed to momentaneous death without any warning. He had no relatives and no additional information could be obtained.

2. A 61 year old female who succumbed to momentaneous death while doing household work. She had not suffered from any atherogenic disease nor complained of any premonitory symptoms.

3. A 33 year old female who succumbed to momentaneous death at home. During the preceding week she had used alcohol in abundance. She had not had any clinical ischaemic heart disease, but there had been congestive heart failure. No information concerning premonitory symptoms was available but a physician was known to have called on her merely one day before her death, the reason for the visit, however remaining unknown.

4. A 53 year old female succumbed to momentaneous death in front of her home door. She had not previously had any ischaemic heart disease or other atherogenic disease. She had not complained either of any particular symptoms during the last month.

5. A 44 year old female succumbed to momentaneous death at home. No ischaemic heart disease had been diagnosed earlier but she had suffered from dyspnoea of effort. During the last week, she had complained of discomfort in the chest, which was not of the effort angina type, and of tiredness and nausea. She had announced her intention to see a doctor for these symptoms.

6. A 55 year old male succumbed to momentaneous death on a beach, after swimming. No history of ischaemic heart disease or premonitory symptoms was available. Instead, he had suffered from obliterative arteriosclerosis of the legs, for which a sympathectomy had been performed 8 years earlier.

Non-autopsied cases

In 47 cases acute ischaemic heart disease was considered to be the cause of death, without confirmation by autopsy. 41 of these were males and 6 were females. Fourteen cases occurred outside Helsinki, two of them abroad. The autopsy rate in the other parts of Finland is much lower than in Helsinki, and this explains why some of the death certificates were signed on fairly feeble grounds.

It is natural that in non-autopsied cases the history of the acute attack becomes most important when the validity of the AIHD diagnosis is considered. Severe chest pain was associated with the fatal attack in 11 male and two female cases. The event was fully momentaneous, without any report of chest pain, in 24 male and four female cases. An atypical attack was reported in three male cases, and in another three male cases there was no accurate information about the attack except for mention of its rapid course.

The prevalence of clinical ischaemic heart disease during life in the non-autopsied cases is presented in Table 2. It seems to be very high among males. No less than 35 out of 41 males had either experienced a myocardial infarction or suffered from angina pectoris during their life. In contrast, only one female had a positive history of previous IHD.

It is usually considered that if a person with a known history of ischaemic heart disease meets with sudden death, IHD is also a highly probable cause of death. Such history was lacking in six male and five female cases. In one male and two female cases, however a typical severe chest pain was reported. In another two male cases a

Table 2. Prevalence of previous myocardial infarction and angina pectoris in non-autopsied cases of sudden coronary death.

Age	Total	History of		No history
		myocard. infarction	angina pectoris	of IHD
Male				
40-49	4	2	1	1
50-59	13	3	8	2
60-63	24	12	9	3
Total	41	17	18	6
Per cent		41.5	43.9	14.6
Female				
55-59	—	—	—	2
60-63	4	—	1	3
Total	6	—	1	5
Per cent		—	16.7	83.3

fresh occurrence of angina pectoris during the last month prior to death supported the diagnosis of AIHD. The case histories for the remaining 3 male and 3 female cases are as follows:

1. A 55 year old man died outside Helsinki, suddenly after swimming. He was single and no relatives or friends could be contacted. No information on previous health or premonitory symptoms was available. The death certificate was signed by a physician at a local hospital, to which the patient was taken after his death.

2. A 63 year old man. The only information available was that death occurred momentarily within 5 minutes, outside Helsinki. The man was a widower and no relatives could be contacted even the physician who signed the death certificate could not give any additional information.

3. A 65 year old man being treated in a psychiatric hospital died outside the hospital. There was no history of previous IHD or any other cardiovascular disease. He underwent a rapid attack with pallor and transpiration, which progressed to death in 45 minutes. There was no information about premonitory symptoms during the last 4 weeks.

4. A 55 year old female died momentarily outside Helsinki. No information on previous diseases or premonitory symptoms could be obtained.

5. A 61 year old female died momentarily outside Helsinki. She had had hypertension, which was not being treated. During the last two weeks she had complained of discomfort in the chest.

6. A 62 year old female died momentarily in the street. She had earlier been treated for hypertension and congestive heart failure. She had not complained of any prodromal symptoms.

Comments

It is generally accepted that ischaemic heart disease is the commonest cause of sudden death. Therefore in many epidemiological studies, sudden unexpected deaths are considered to be due to AIHD if no other obvious cause of death can be pointed to. It must be emphasized, however that autopsy is the only means to demonstrate the cause of sudden death with any certainty.

Most community studies in the U.S.A. and Great Britain have reported fairly low autopsy rates. Only 3 out of 45 SCD cases were autopsied in the Tecumseh study (Chiang et al. 1970). The autopsy rate was 30 per cent in Belfast (McNeilly 1967) and 64 per cent in the Edinburgh Community Study (Armstrong et al. 1972). Higher autopsy rates have been reported from Sweden (Slevens 1963 Fodor 1969 Wiklund 1971).

A high autopsy rate (80.3 per cent) was arrived at in the present study which should provide a certain guarantee that the majority of these cases truly represent AIHD. In fact, the autopsy rate seems to be among the highest reported.

A recent myocardial infarct or a fresh thrombus was found in a high percentage in spite of the short duration of the attack. This is in fact strange, although many previous autopsy studies have indicated the same. It can be speculated that the initial phase of the fatal attack has been silent or that the victim has not reported such symptoms as he may have had.

In contrast to the validity of a fresh myocardial infarct, that of acute ischaemic changes may perhaps be contested. Autolysis causes similar changes, neither can its role be excluded in this material. On the other hand, changes suggesting acute ischaemia were the only basis for diagnosis in a minority of cases only.

It is not unexpected that in many cases no acute changes whatsoever were found at autopsy. The percentage of negative post mortem findings should actually be even higher on the basis of present knowledge as to the time needed for the development of visible changes. Scars from old myocardial infarcts and advanced coronary arteriosclerosis were found in high percentage and these at least indicate that the person has really had IHD during his life although the contribution of these findings to the acute fatal event cannot be demonstrated.

The degree to which occlusive coronary arteriosclerosis contributes to sudden death is not established. Some degree is an almost uniform finding in the middle-aged or elderly population and it is not possible to point to any critical degree of occlusion (Roberts 1972).

In the present study it was considered, quite arbitrarily that a severe coronary arteriosclerosis means that the lumen of at least one of the main coronary arteries is totally occluded. This finding was considered to justify the entry of AIHD as a cause of sudden death. In cases with coronary arteriosclerosis of a lower grade, additional support was sought from the case history. This was also obtained in the majority of cases.

After all, there remained only 6 autopsied cases in which the cause of death remained uncertain. However this group only constitutes 3.1 per cent of all autopsied cases and 2.5 per cent of the whole SCD group.

While the diagnosis of sudden coronary death may remain uncertain at autopsy the basis for diagnosis is even weaker in non-autopsied cases. Acute chest pain in connection with the fatal attack is considered a strong support for the diagnosis of AIHD but the death is often quite momentaneous without any attendant chest pain. Wiklund (1971) has recently shown that 10 per cent of sudden fatal attacks with acute chest pain or dyspnoea were caused by diseases other than IHD.

A high prevalence of previous symptomatic IHD was found in the non-autopsied series. According to the present understanding, when a person with known IHD meets with sudden death, this event is due to AIHD with a high probability rating. There remained only a few cases of momentaneous death without any additional support for the diagnosis.

It must be realized that no general agreement exists concerning the diagnostic criteria of SCD. The cause of death can be deter-

mined with a higher or lower probability rating, which varies from case to case. A high autopsy rate, no doubt, improves the level of validity of the diagnosis, but even a hundred per cent autopsy rate would not be able to guarantee correct diagnosis in one-hundred per cent of cases. Against this background the validity of the diagnosis in the present series of SCD can be considered satisfactory.

Summary

192 of the total 239 SCD cases were autopsied (80.3 per cent). The autopsy rate was 79.5 per cent for males and 84.6 per cent for females. This seems to be a very high rate by international standards.

In spite of the short duration of the attack a recent myocardial infarction or a fresh thrombus was found in a high proportion of cases in 40 per cent of male and 30 per cent of female cases. In 92 per cent of male and 79 per cent of female autopsied cases, the autopsy findings were considered as speaking strongly in favour of AIHD or at least of severe IHD.

The diagnosis of AIHD was supported by previous symptomatic IHD in 36 out of 47 non-autopsied cases.

When all the relevant information was taken into account, the cause of death was considered to be unreliably fixed in a few cases only.

4 DEMOGRAPHIC CHARACTERISTICS

Marital status

The patient's marital status is always entered in the hospital records and also in the death certificates. Therefore, this information could almost invariably be obtained. It is clearly evident from official vital statistics that the distribution by marital status changes markedly with age. In addition to the usual tabulation, it was therefore considered nec-

Age	Sudden deaths					Non-sudden deaths					Survivors					All										
	Total			M		W		Total			M		W		Total			S		M		D		W		
	S	M	W	M	W	M	W	S	M	W	S	M	W	S	M	W	S	M	W	S	M	D	W			
—19	43	6	34	3	—	31	2	23	5	1	148	15	117	35	2	—	234	27	179	23	3	—	—	—		
20—39	3	6	56	7	4	72	6	56	7	3	239	12	182	21	14	—	396	28	368	42	25	—	—	—		
40—59	82	6	62	4	8	72	4	35	9	4	148	7	115	12	14	—	316	20	235	11	30	—	—	—		
Total	128	20	153	14	12	175	12	124	21	8	336	34	434	48	30	—	946	73	720	98	53	—	—	—		
Per cent	18	27	7	6	—	7	77	11	4	—	6	79	9	6	—	—	8	76	10	6	—	—	—	—		
$\chi^2=2.76$ N.S.D.																										
	Females					Males					Females					Males										
—19	4	—	3	1	1	8	2	3	—	—	24	3	15	4	2	—	24	5	20	6	3	—	—	—		
20—39	18	1	9	4	5	19	3	8	3	5	79	11	44	8	14	—	120	16	83	15	28	—	—	—		
40—59	16	1	9	3	3	40	13	17	2	8	83	3	38	12	23	—	138	1	64	18	25	—	—	—		
Total	38	2	20	8	9	64	17	28	5	14	186	19	99	24	34	—	312	43	147	39	54	—	—	—		
Per cent	8	51	21	23	—	27	44	8	21	—	10	61	12	28	—	—	13	47	13	17	—	—	—	—		
$\chi^2=17.32^{**}$																										
52 cases (29 male 13 female) of unexplained death included.																										
S=Single, M=Married, D=Divorced, W=Widowed																										

152 cases (39 male 13 female) of unWitnessed death included. $\chi^2 = 3.76$ N.S.D. S=Single, M=Married, D=Divorced, W=Widowed

essary to investigate the potential association of marital status with sudden death, non sudden death and the survival from an AIHD attack in a manner enabling the effect of age to be eliminated. An age adjustment process was accordingly applied. The method and mathematical treatment are laid out in Chapter III. 4.

Results

Table 9 presents the marital status distribution in the three comparison groups, SCD NSCD and SURV. It can be seen that in both sexes a slightly higher number of married persons than expected survived their AIHD attack. Both single and divorced persons were slightly under represented among the survivors. A striking accumulation of single females is found in the NSCD group. The differences were statistically significant among females, but non-significant among males.

Fig 8 presents the three comparison groups, SCD, NSCD and SURV in different marital status categories in the form of age-adjusted observed-to-expected ratios. The ratio equals unity if the observed number of cases coincides with the number expected upon age adjustment. The group of un witnessed deaths has been excluded from the figure, although it was included in the calculations. This group had higher numbers than expected in all categories other than that of married persons, and this is fully plausible.

The over-all picture of distribution by marital status somewhat changes after age adjustment, as can be found when Table 9 is compared to Fig 8. There are more married people and less single people than expected among the survivors of both sexes. The divorced persons, again, seem to be more numerous than expected among survivors. Sudden death seems to be most often the fate of single males and widowed females. The age adjustment abolished the

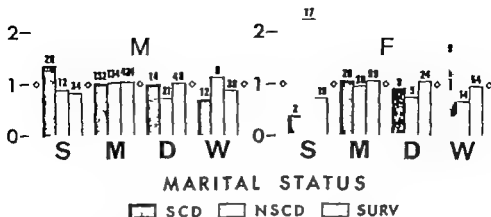


Fig. 8. Preponderance of persons belonging to SCD NSCD and SURV groups, expressed as age-adjusted observed-to-expected ratios, in different marital status categories. S = Single, M = Married, D = Divorced, W = Widowed. Figures over each column number of observed cases.

statistical significance which was found among females the differences are now non-significant in both sexes.

Attempts of several kinds were made to account for the effect of the group of unwitnessed death. In Fig 8 this group was included in the calculations, although it was not presented in the diagrams. In another analysis this group was excluded and only the actual comparison groups, SCD NSCD and SURV were considered. Finally the unwitnessed death cases were added to the sudden and non-sudden death cases in proportion to the size of these groups. By all methods the result subsequent to age adjustment was the same the differences in marital status distribution between the SCD NSCD and SURV groups were non-significant.

Comments

Several previous studies have suggested a certain over mortality in IHD among non-married persons giving rise to much speculation.

A slight trend in the same direction as mentioned above is discernible in the present study. It must be realized that in a study of the present kind various bias factors easily cause pseudo-correlations lacking any actual

connection with the marital status itself. It is to be fully expected, by definition, that marital status categories other than that of married persons are over represented in the group of unwitnessed deaths. Although this group is relatively small, it still impedes the comparison of the SCD and NSCD groups. A significant bias may be incurred if the effect of age is not taken into account, because the relative proportion of widowed and divorced persons increases strongly in the older age groups.

Finally it has to be emphasized that married persons constitute a great majority of all cases. In Fig 8, the distribution of the comparison groups is most uniform in this largest group. In the other marital status categories, on the other hand, the small numbers may be responsible for seemingly remarkable differences, which turn out to be non-significant.

Summary

After age adjustment no particular marital status category was significantly associated with any one of the comparison groups SCD NSCD or SURV. Obvious bias factors were demonstrated and attempts were made to eliminate their influence.

Table 10 Distribution of acute ischaemic heart disease patients by age at comparison groups by age sex and social class (I-V)

Age	Sudden deaths				Non sudden deaths				Survivors				All			
	Total		I		Total		I		Total		I		Total		I	
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Males																
45-49	41	6	10	18	7	31	3	7	14	7	150	22	58	41	20	38
50-54	2	14	18	30	10	73	15	18	33	9	239	51	45	107	22	56
55-59	82	1	17	37	16	72	18	13	27	12	149	31	22	70	7	53
Total	185	32	45	85	33	16	36	38	74	28	538	115	106	218	9	149
Per cent	16	23	44	17		20	22	43	18		21	9	44	15		18
Females																
45-49	4	—	—	3	1	3	—	1	3	—	26	4	1	13	9	10
50-54	19	—	5	6	6	10	4	3	7	3	77	3	18	41	14	26
55-59	15	—	2	12	1	41	4	5	21	11	23	9	22	37	5	43
Total	38	—	7	21	10	54	8	11	30	14	106	16	42	90	48	78
Per cent	0	18	26	6		13	17	48	23		8	21	46	34		25

p = 3.27 N.S.D. p = 3.94 N.S.D.

Social class

The social classification used in Finland is presented in Chapter III 3. The census data of 1965 for Helsinki reveal that the social class distribution changes markedly with age. It was therefore considered necessary to analyse the association of social class with the comparison groups also by means of age-adjusted ratios, as was done in the foregoing concerning marital status.

A quite considerable percentage of all patients with AIHD had retired from work prior to their attack, i.e. no less than 31 per cent of males and 37 per cent of females. Therefore, analysis concerning social class was also performed for non retired and retired persons separately.

Results

Table 10 presents the social class distribution in the comparison groups, SCD NSCD and SURV. Among males the distribution is fairly uniform in all comparison groups. Among females it can be noted that there were no cases of sudden death in the highest social class. The differences observed were statistically non-significant.

Fig. 11 presents, in the form of age-adjusted observed to-expected ratios, the distributions of the three comparison groups by four social classes. The diagrams on top refer to those who were still working when the attack occurred in the centre the retired persons have been shown, and at the bottom the findings concerning both groups together can be seen.

The picture is seen to be largely the same for all cases on one hand and for working subjects on the other. In contrast, the retired persons display a different picture.

Among males sudden death is found to be less frequent and survival more frequent than expected in the highest social class I. The opposite is true of the lowest social class IV. The proportion of sudden deaths

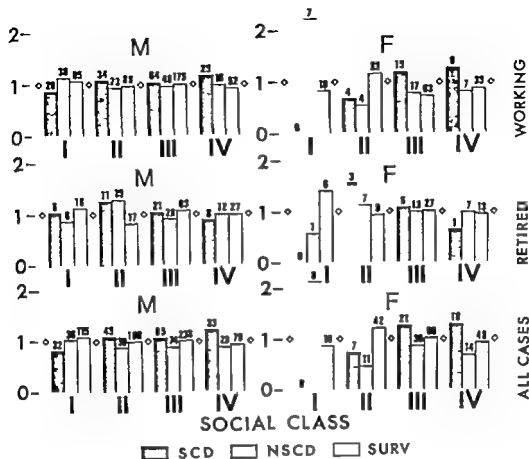


Fig. 9 Preponderance of cases belonging to SCD NSCD and SURV groups, expressed as age-adjusted observed-to-expected ratios, in different social classes. Working (top), retired (centre) and all cases (bottom). I = highest, IV = lowest social class. Figures over each column number of observed cases.

appears to increase fairly uniformly from the highest to the lowest social class.

Among females no such lucid picture can be seen. It is notable that there were no sudden death cases in the highest social class. The proportion of sudden deaths actually increases, also among females, from the highest to the lowest social class. The proportion of survivors, however is lower than expected in both social classes I and IV. It should be noted, though, that the ratios are based on small numbers.

Subsequent to age adjustment there were no statistically significant differences among either males or females.

Comments

The association between social class and incidence of IHD has been an object of several studies over the last 20 years (Marks 1957, Antanovsky 1968, Hinkle et al. 1968). The differences observed have usually been attributed to differences of the average level of physical activity although, without doubt, other differences of importance also exist in the mode of life between different social classes. It seems appropriate to assume that in recent years these differences have diminished and the average on-the-job physical activity commonly considered as explaining

Tbl 11 Prevalence of previous symptomatic ischemic heart disease (IHD) and myocardial infarction in different comparison groups.

Age	Sudden deaths				Non-sudden deaths				Survivors				All	
	Tot	Data av	Myoa. inf.	IHD	Tot	Data av	Myoa. inf.	IHD	Tot	Data av	Myoa. inf.	IHD	Per cent	Per cent
Males														
40-49	44	37	13	31	29	13	15	53	150	149	28	79	53	54
50-59	73	66	25	73	68	34	53	71	239	271	84	184	65	70
60-69	83	77	30	77	66	33	53	80	149	147	54	117	80	80
Total	200	180	69	133	176	143	80	131	538	535	176	350	66	70
Per cent			28				49				33			
$\chi^2 = 13.39^*$ (myocardial infarctions) $\chi^2 = 8.86$ (total IHD)														
Females														
40-49	4	3	1	4	4	3	3	33	26	26	4	18	68	70
50-59	19	16	5	19	17	2	14	82	78	78	25	54	69	69
60-69	6	18	5	6	39	16	30	77	84	94	25	72	77	78
Total	29	34	11	18	64	60	21	47	196	196	54	144	73	71
Per cent			23				25				29			
$\chi^2 = 0.74$ N.S.D. (myocardial infarctions) $\chi^2 = 5.88$ N.S.D. (total IHD)														

mal 13 female) of unexplained death included.
 co of previous IHD

IHD = Myocardial infarction and/or angina pectoris

the lower incidence of IHD in lower social classes, is continuously decreasing. The role of physical activity will be discussed in detail later.

Data on the relationship between social class and sudden coronary death are scarce. When the results of the present study are considered, the most notable finding is that there were no statistically significant differences between the different social classes. Nevertheless, a slight tendency seems to exist in that sudden death is more frequent in the lower social classes, in which the prognosis seems to be less favourable. This finding, though not significant, corresponds to those studies cited from Helsinki by Gorbатов et al. (1971).

The picture was closely similar in the entire material and in the sub-series of non-retired persons, whereas retired persons, treated as a series of their own, presented a somewhat different picture. This may however be based on small numbers alone. It hardly warrants any conclusions, especially as these differences, too, are non-significant. It is also due to the small numbers that the differences among females, while appearing quite striking in the diagram, do not reach any significant level.

Summary

Social class was not significantly associated with any one of the three comparison groups, SCD NSCD or SURV. However a slight tendency seemed to exist in that sudden death was more often the fate in the lowest social classes and that it was correspondingly less frequent in the upper classes.

5 PREVALENCE OF CERTAIN PREVIOUS DISEASES AND RISK FACTORS

The same risk factors which predispose to all manifestations of IHD seem to be associated with sudden coronary death, too, although their predictive power may be dif-

ferent in different manifestations of IHD. The symptomatic clinical ischaemic heart disease itself, i.e., a myocardial infarction or angina pectoris, involves a definite excessive risk of sudden death. It will therefore also be dealt with in this connection. While many risk factors seem to be independent, the combination of several of them mostly multiplies the risk (Stamler 1967 Epstein 1968). The whole spectrum of risk factors and previous diseases can be observed by methods of multifactorial analysis, as will be presented later. In the following the prevalence of some major diseases and risk factors will be separately analysed.

Symptomatic ischaemic heart disease

The history of myocardial infarction or angina pectoris was based on information gained from the patients or their relatives and, whenever possible, confirmed by hospital records. Both confirmed and non-confirmed episodes of a previous myocardial infarction were accepted. 88 per cent of the episodes could be confirmed, and in this respect there were no significant differences between the groups.

Results

Table 11 presents the prevalence of myocardial infarction and of the total of previously diagnosed symptomatic ischaemic heart disease, including both myocardial infarction and angina pectoris.

88 per cent of male and 81 per cent of female patients with AIHD were reported to have had a myocardial infarction in the past. The prevalences were higher for males than for females in all comparison groups and increased with age in both sexes. In both sexes the lowest prevalence was seen in the SURV group and the highest in the NSCD group. The SCD group was intermediate between these. The differences were statistically significant among males but non-

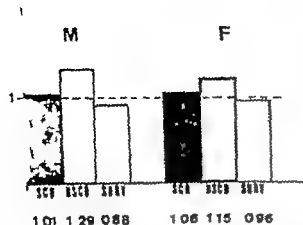


Fig 10 Preponderance of patients with previous myocardial infarction in SCD NSCD and SURV groups, expressed as observed to-expected ratios.

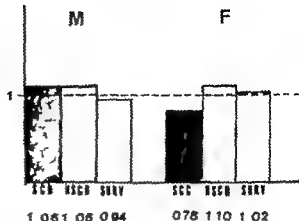


Fig 11 Preponderance of patients with symptomatic ischaemic heart disease (myocardial infarction and/or angina pectoris) in SCD NSCD and SURV groups, expressed as observed to-expected ratios.

significant among females. The distribution of patients with a previous myocardial infarction in the three comparison groups is presented in Fig. 10 as observed-to-expected ratios, separately for males and for females. The accumulation of these patients in the NSCD group is clearly evident.

The sex difference disappears when the prevalence of total symptomatic IHD is considered to be 70 per cent for males and 71 per cent for females with AIHD. Among males, the prevalence of symptomatic IHD increased with age whereas that of females remained fairly constant in all age groups. The differences between the comparison groups reached an almost significant level among males. This is due to the lower prevalence in the SURV group compared to the SCD and NSCD groups, in which the prevalences were nearly equal. Among females, again, the prevalence of symptomatic IHD was lowest in the SCD group and highest in the NSCD group but with no significant differences because of the small numbers. The distribution of patients with a diagnosed IHD in the three comparison groups is again presented as observed to-expected ratios in Fig. 11.

Comments

It is not unexpected that a great proportion of patients with AIHD in a cross-sectional study already suffer from symptomatic IHD. The lower frequency of previous myocardial infarction among survivors, compared to the SCD and NSCD groups, is in accordance with the well known higher mortality in recurrent attacks compared to the first attacks. In both sexes the highest prevalence was found in the NSCD group. This finding too, conforms with several previous studies, which suggest that the mode of death in recurrent attacks is delayed rather than sudden (Achor et al. 1956, Weiss 1956, Liebow and Badger 1963, Hagström et al. 1967, Badger and Liebow 1968, Rissanen et al. 1971).

The prevalence of previous myocardial infarction found in the SCD group seems to be slightly higher than in previous studies, which report prevalences of around 30 per cent (Banton and Peterson 1963, Fulton et al. 1969). As a conclusion, it can be stated that a history of myocardial infarction seems to increase the risk of death, but the death is more often delayed than sudden.

A somewhat different picture is obtained when the total symptomatic IHD is taken into consideration. Previous studies have reported prevalences of 40 to 59 per cent for total symptomatic IHD among those who experience sudden coronary death (Bainton and Peterson 1963 Myerburg and Davis 1964 Kuller et al. 1966 a, McNeillly 1967, Fulton et al. 1969 Stamler et al. 1969 Chiang et al. 1970 Romo et al. 1971). The prevalence of total IHD in the present series seems very high. This concerns all patients with AIHD. In the SCD group, among males, the prevalence of total IHD is as high as 74 per cent among females it is definitely lower namely 56 per cent. In the two other comparison groups the male prominence disappears when the prevalence figures of myocardial infarction and angina pectoris are combined. The prevalence of total IHD is in fact higher for females than for males in the NSCD and SURV groups. This is due to the higher frequency of angina pectoris among females in these groups.

In males, the prevalence figures of total IHD can be interpreted in that a history of IHD increases the risk of dying in an AIHD attack, even though no influence on the mode of death can be seen. Among females the prevalence figures are more difficult to interpret.

Summary

The prevalence of previous myocardial infarction, and that of symptomatic total IHD which includes angina pectoris, is stated in all comparison groups. Among both sexes a history of myocardial infarction was more often associated with death than with survival of an AIHD attack, but the death was more often delayed than sudden. No further conclusion can be drawn from the prevalence figures of total IHD. The prevalences of myocardial infarction and total IHD are both higher than those usually reported.

Hypertension

Diagnosis of hypertension was based on the information given by the patients or their relatives and, whenever possible, on hospital records. 89 per cent of all reported instances of hypertension were considered confirmed and the remaining 11 per cent unconfirmed. No significant differences were noted between the comparison groups in the percent ages of confirmed and unconfirmed hypertension.

Results

Table 12 shows the prevalence of reported hypertension in the comparison groups. The differences between the groups were almost significant among males but non-significant among females.

The frequency of hypertension appears to be high in both sexes and all ages. It is definitely higher in females than in males and increases with age in both sexes. The only exception is the SCD group of males, where the highest prevalence is found in the age group of 60-69 years.

When the groups with different prognosis are compared, a slight but definite trend can be seen. The prevalence of hypertension is in both sexes lowest in the SURV group, increases in the NSCD group and is highest in the SCD group. This trend is more marked among males and only minimal among females. The distribution of patients with reported hypertension in the three comparison groups is presented in Fig 12, as observed-to-expected ratios.

Comments

The high frequency of hypertension in both sexes elicited in the present study parallels those in several previous studies of AIHD. Although there are only small differences between groups, a distinct trend of highest prevalence in the SCD group and lowest

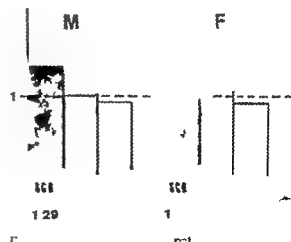
4. An increase in the rate of environmental degradation.

Age	Prevalence of hypertension				Sudden deaths				Non-sudden deaths				Survivors				All			
	T	W	D	Per tension cent	T	W	D	Per tension cent	Total	D	W	Per tension cent	Total	D	W	Per tension cent	Total	D	W	Per tension cent
40-49	44	25	9	25.7	31	27	3	11.1	150	140	29	19.5	256	214	41	19.5	256	214	41	19.5
50-59	4	65	23	30.4	72	63	17	26.2	239	237	47	19.8	397	360	37	23.0	397	360	37	23.0
60-65	21	7	22	18.4	72	63	17	26.2	149	140	39	26.2	320	295	79	26.8	320	295	79	26.8
Total	69	97	54	29.5	176	157	37	23.6	538	535	115	21.5	953	878	207	23.6	953	878	207	23.6
	$\chi^2 = 6.09$																			
	Female																			
40-49	4	2	—	0	4	4	3	15.0	—	—	8	30.8	25	25	11	32.3	25	25	11	32.3
50-59	19	16	7	46.7	19	16	6	37.5	78	78	35	44.9	170	111	48	43.2	170	111	48	43.2
60-65	16	14	9	64.3	41	34	18	47.1	94	84	43	47.9	159	148	73	49.2	159	148	73	49.2
Total	39	32	16	50.0	64	54	25	46.3	196	186	86	44.4	314	294	132	45.2	314	294	132	45.2
	$\chi^2 = 0.33$ N.S.D.																			

prevalence in the SURV group is noted, which is consistent e.g. with the results of two prominent prospective studies, those of Framingham (Kannel et al. 1971) and Tecumseh (Chiang et al. 1970).

It must be realized that the method used is only a rough estimate of the presence or absence of hypertension. A substantial percentage of hypertensive persons are not aware of their disease. Concepts as to what is considered hypertension, as well as indications for antihypertensive treatment, vary greatly even among physicians. There was also no possibility to take into account the level of blood pressure or the influence of treatment. These obvious drawbacks, common to all retrospective studies impede the evaluation of the importance of hypertension regarding the course of an AIHD attack, in a study of the present kind. It is likely that diagnosed hypertension which fails to be recorded most often occurs in the SCD group. It follows that the association of hypertension with SCD which may be found, is in fact minimized.

An important and possibly significant factor may be, moreover, the role of certain antihypertensive medicines potentially associated with sudden death, independent of the blood pressure level. This especially con-



cerns diuretics and the common sequelae of their use, hypokalaemia and disturbances of the heart rhythm. Digitalis and rauwolfia alkaloids, often used together with diuretics, may be speculated as constituting a potentially dangerous combination for some persons at least. In fact, the use of diuretics was more frequent in the SCD group (25 per cent of all these patients) than in the SURV group (12 per cent). The difference was highly significant. However there was no significant difference between the SCD and NSCD groups (24 per cent). The use of digitalis was also more common in the SCD than in the SURV group.

The importance of this finding cannot be definitely judged. Its most likely cause is the poorer condition of persons who are subsequently going to die suddenly causing a more frequent use of medicines such as digitalis and diuretics. However the possibility remains that these medicines may play an independent role as causative factors by predisposing to sudden death.

Summary

The prevalence of hypertension on the whole was found to be high in patients with AIHD. In both sexes, the prevalence was lowest in the SURV group and highest in the SCD group.

Other cardiovascular diseases

Other cardiovascular diseases, excluding symptomatic ischaemic heart disease and hypertension, constitute in this study a heterogeneous group of diseases or symptoms, which are mostly closely related to atherosclerosis or ischaemic heart disease itself. The diseases comprise e.g. a stroke, and the symptoms include such as congestive heart failure or various disturbances of rhythm, which in fact in most cases are also manifestations of IHD.

Results

A stroke has mainly affected persons who already suffered from symptomatic IHD or hypertension. The whole group of AIHD patients contains only 20 persons (16 male and 4 female) with a history of stroke who were reportedly free from the two diseases mentioned. Of them, four males and one female belonged to the SCD group.

35.1 per cent of all patients whose use of medicines was known used digitalis preparations. The corresponding percentages in the SCD NSCD and SURV groups were 38.8 52.2 and 28.9 respectively. These differences are highly significant. If use of digitalis is considered an index of heart failure although an unreliable one, it seems as if the heart failure were slightly more frequent in the SCD group and markedly more frequent in the NSCD group than in the whole series of AIHD patients. Therefore, heart failure seems to be associated with death in an AIHD attack, and most often with delayed death. In the majority of cases undoubtedly the basis of heart failure was either IHD itself or hypertension. There were, however 39 patients (27 males and 12 females) with a history of congestive heart failure who were reportedly free from the two diseases mentioned. Of them, 5 males and 2 females belonged to the SCD group. This does not mean that these patients would have been free of these diseases, which had not been diagnosed, however.

Only a few examples of valvular congenital or pulmonary heart disease were reported as previous diseases. Special attention was paid to aortic stenosis, which is also known to be responsible for sudden fatalities. Only two cases of aortic stenosis were reported in the SCD group, but in both cases autopsy revealed a fresh myocardial infarction, whereby the contribution of the valvular lesion to the sudden deaths in question remained uncertain.

02 cases (39 male 13 female) of unwitnessed death included.

Table 12. Prevalence of diabetes mellitus in different comparison groups

Age	Sudden de the			Non-sudden de the			Survivors			All		
	Total	D	Diab.	Total	Data	Diab.	Total	Data	Diab.	Total	Data	Diab.
		av	per cent		av	per cent		av	per cent		av	per cent
—49	44	36	82	31	28	4	150	150	10	236	218	18
50—59	72	66	91	72	68	10	239	238	25	397	374	41
60—69	53	49	92	72	67	8	149	148	23	220	208	41
Total	170	161	94	176	163	13	538	537	58	953	892	100
—49	4	3	—	4	4	2	28	20	—	33	23	2
50—59	19	16	83	19	16	6	88	78	10	170	132	17
60—69	16	13	81	41	38	12	94	94	10	159	152	28
Total	39	32	82	64	58	10	199	190	20	314	298	47

Summary

The group of other cardiovascular diseases in the present study represents a heterogeneous group of diseases or symptoms of varying significance. Largest contributions were those of heart failure and stroke, which, however usually displayed concomitant symptomatic IHD or hypertension. The independent contribution of the diseases in this group was small.

Diabetes mellitus

The history of diabetes mellitus was based on information obtained from the patients or their relatives. Confirmation was sought from hospital records. The patients with a positive history of diabetes mellitus present a wide variation in severity and duration of the disease and the treatment which they needed varied from insulin treatment to a restricted diet.

Results

The prevalence of diabetes mellitus in the comparison groups is presented in Table 13. Taking all patients into consideration, the prevalence seems to be somewhat higher among females (15.6 per cent) than among males (11.2 per cent) increasing with age in both sexes.

Among males the differences between groups are small and non-significant. The prevalence was lowest, 9.4 per cent, in the SCD group.

Among females the prevalence is definitely highest in the NSCD group (34.5 per cent), and, therefore, the differences between groups among females reach a highly significant level. The prevalences in the SCD and SURV groups were more closely equal, 14.7 and 10.1 per cent, respectively.

Comments

The prevalences given in this study for the SCD group, 9.4 per cent for males and 14.7 per cent for females, are on a level with those reported earlier in studies on sudden coronary death. A history of diabetes mellitus was obtained in 11.6 per cent of SCD cases in Baltimore (Kuller et al. 1967). In Tecumseh, eight out of 43 persons (18.7 per cent) who succumbed to sudden death due to AIHD had diabetes mellitus (Chiang et al. 1970). The prevalence of diabetes mellitus was 10.6 per cent among 964 medically unattended death cases in Stockholm. Diabetes seemed to have no association with the duration of the fatal attack (Wiklund 1971). In the Framingham as well as the Tecumseh study diabetes mellitus seems to be associated with death in AIHD (Kannel et al. 1957 a, Epstein and Ostrander 1971).

The same trend, though very slight in degree can be seen in the present study. In both sexes the prevalence of diabetes mellitus was lower among survivors than among all patients with AIHD. On the other hand, death seems to be delayed rather than sudden the difference noted in females is striking indeed, although the numbers are small. Diabetes mellitus seems to be an unfavourable concomitant disease when an AIHD attack occurs, but it is not a predictor of sudden death. The prevalences in all three comparison groups are higher than those usually reported in populations at large. This, too, is consistent with previous studies and with the well-known atherogenic effect of diabetes mellitus.

Summary

The prevalence of diabetes mellitus was fairly high in all groups of AIHD. A history of diabetes mellitus was more often obtained from persons who died in the AIHD attack, and the highest prevalences were noted in the NSCD group, especially among females.

Patients without any major cardiovascular disease or diabetes mellitus

A certain number of persons with AIHD were obviously free from clinical symptoms of any major disease related to arteriosclerosis and in this respect they could be considered healthy at the moment of their acute attack. Patients with a history of any one of the following diseases were excluded from the consideration: symptomatic IHD, hypertension, stroke, other cardiovascular diseases, and diabetes mellitus. The prevalence of these diseases is presented earlier in this chapter.

Results

Table 14 shows the number of persons reportedly free from the above-mentioned diseases in the comparison groups. Of all patients with AIHD 20 per cent of males, but only 9 per cent of females, were considered healthy. Among males the proportion of healthy persons decreased with age, while among females no clear effect of age could be noticed.

Among males the proportion of healthy persons was greatest in the SURV group (24 per cent) compared to the SCD and NSCD groups in which their proportion was equal (13 per cent). This difference between the male groups reached a highly significant level. Among females the differences between the three comparison groups were non-significant and the proportion of healthy persons was greatest in the SCD group (12 per cent).

The distribution of persons considered healthy in the three comparison groups is presented also in Fig. 13. The accumulation of healthy persons in the SURV group in males is clearly evident in the figure. Among females, the ratios were higher than expected both in the SCD and SURV groups but it must be realized that the number of healthy females was very small indeed.

diabetes mellitus in different comparison groups.

Age	Sudden deaths			Non sudden deaths			Survivors			All			
	Total	D to Healthy	Per cent	Tal	Data	Healthy Per cent	Total	Data	Healthy Per cent	Total	Data	Healthy Per cent	
—44	44	37	84	31	29	94	130	149	54	34	336	313	72
	3	3	100	73	68	93	338	337	33	23	397	373	65
	63	77	122	72	66	92	149	147	19	13	320	294	35
	100	180	180	176	163	93	538	533	100	4	955	878	175
45—59	4	3	75	4	4	100	6	3	—	0	35	23	1
	18	16	89	18	17	94	78	78	13	17	150	112	15
	18	15	83	41	39	95	94	94	7	7	159	153	12
	38	34	89	84	80	95	198	197	100	10	314	298	5
60—65	18	15	83	41	39	95	94	94	7	7	159	153	12
	38	34	89	84	80	95	198	197	100	10	314	298	5
	18	15	83	41	39	95	94	94	7	7	159	153	12
	38	34	89	84	80	95	198	197	100	10	314	298	5
Total	100	180	180	176	163	93	538	533	100	4	955	878	175
	43	37	86	35	31	89	130	149	54	34	336	313	72
	63	77	122	72	66	92	149	147	19	13	320	294	35
	100	180	180	176	163	93	538	533	100	4	955	878	175

51. 59 male 13 female) of unspecified death included.

Comments

It is usually thought that AIHD often appears in a previously healthy person without any premonition. On the other hand, several studies have shown that the majority of persons who have an AIHD attack or succumb to sudden cardiac death have suffered before from symptomatic IHD or other diseases related to atherosclerosis.

Relatively few persons with AIHD could be considered healthy prior to the attack. In the present study Among males this was even more infrequent among those who died by an AIHD attack. It seems as though healthy persons had a better prognosis than others in the event of an AIHD attack.

Among females only a few persons could be considered healthy prior to the attack and the small numbers hardly warrant any conclusions concerning their distribution in the comparison groups.

In the SCD group only 13 per cent of males and 12 per cent of females were free from any major atherosclerotic disease. From the practical point of view this fact might possess great importance. It is generally accepted that preventive measures should be

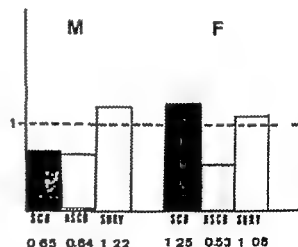


Fig 12 Preponderance of patients without any major cardiovascular disease or diabetes mellitus in SCD, NSCD and SURV groups expressed as observed to-expected ratios

Tabl 14 Patient without a w major cardiovascular disease or diabetes mellitus in different comparison groups.

Age	Sudden deaths				Non-sudden deaths				Survivors				All's	
	Total	Data	Healthy	Per cent	Total	Data	Healthy	Per cent	Total	Data	Healthy	Per cent	Total	Healthy
Males														
—19	44	27	9	4	31	28	9	31	150	148	84	56	238	213
20—39	73	66	7	11	73	65	4	6	239	237	63	22	397	373
40—59	83	77	7	9	73	65	8	12	149	147	19	13	250	234
60—65	400	180	23	13	16	163	1	13	538	533	126	4	863	846
Total														
Females														
—19	4	3	1	23	4	4	—	0	46	25	—	0	35	33
20—39	19	16	1	6	19	17	—	0	78	72	13	17	120	113
40—59	16	15	2	13	41	39	3	8	94	84	7	7	168	153
60—65	29	34	4	12	64	60	3	3	198	197	30	10	314	298
Total														

51 50 (28 mal 12 female) of unwitnessed death included.

Comments

It is usually thought that AIHD often appears in a previously healthy person without any premonition. On the other hand, several studies have shown that the majority of persons who have an AIHD attack or succumb to sudden cardiac death have suffered before from symptomatic IHD or other diseases related to atherosclerosis.

Relatively few persons with AIHD could be considered healthy prior to the attack in the present study. Among males this was even more infrequent among those who died by an AIHD attack. It seems as though healthy persons had a better prognosis than others in the event of an AIHD attack.

Among females only a few persons could be considered healthy prior to the attack and the small numbers hardly warrant any conclusions concerning their distribution in the comparison groups.

In the SCD group only 13 per cent of males and 12 per cent of females were free from any major atherosclerotic disease. From the practical point of view this fact might possess great importance. It is generally accepted that preventive measures should be

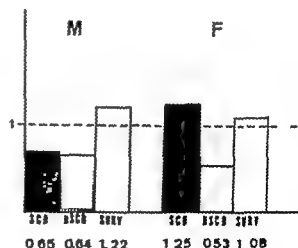


Fig 13 Preponderance of patients without any major cardiovascular disease or diabetes mellitus in SCD NSCD and SURV groups, expressed as observed to-expected ratios.

No clear association between age and body mass index could be found. The lowest means in both sexes appeared in the NSCD group. Among males, the differences between the comparison groups reached a statistically significant level. This was mainly due to the difference between the NSCD and SURV groups. Among females no significant differences appeared.

Comments

No general consensus of opinion exists concerning the demarcation of normal weight and overweight, nor concerning the best index of an individual's obesity although a great range of calculating procedures have been used. A recent study comparing various methods and calculations applied before has led to the conclusion that the body mass index, i.e. weight over height-squared, corresponds best to a person's true obesity provided the basis of estimation consists of weight and height figures (Keys et al. 1972). This index was chosen in the present study.

In a study of this kind, the reliability of the weight and height data is inevitably variable. The figures given by the relatives, or even by the patients themselves, may be wrong. Moreover the data gathered from hospital records do not always reflect the true level of weight in normal circumstances. Autopsy data are not fully comparable with those obtained from other sources. This is an unavoidable methodological shortcoming in a retrospective study.

In the present study the body mass index was definitely lowest in the NSCD group while the means both in the SURV and in the SCD groups were on the same level. Obesity therefore is associated with survival, but if with a fatal attack then rather with sudden death.

Summary

The relative weight of patients with AIHD

was determined by calculating a body mass index. The mean values in the SCD group were on the same level as in the SURV group but higher than in the NSCD group. The differences between the groups were significant among males but non-significant among females.

Smoking habit

The history of smoking and, if positive, the daily consumption of cigarettes of every patient were elicited by questioning. The categories with regard to smoking are defined as has been stated before, Chapter III. 2.

Results

Table 16 is a compilation of the smoking habit data in the comparison groups.

Current smokers constituted 66 per cent of all male and 40 per cent of all female patients with AIHD. Half of all female patients, but only ten per cent of all male patients were non-smokers. The relative proportion of current smokers decreased with age in both sexes.

When smoking habits in the different comparison groups are considered, it can be seen that there are only slight differences between the groups among males. The proportion of current smokers was lowest in the SCD group, which correspondingly had the highest percentage of non-smokers. The NSCD and SURV groups showed largely similar distributions within the different smoking categories. The differences were non-significant on the whole.

Somewhat greater differences seem to occur among females, but they too are non-significant owing to the small numbers. In contrast to the males, the proportion of current smokers was highest in the SCD group with the correspondingly lowest percentage of non-smokers.

Table 1. Death in relation to current smokers among acute ischaemic heart disease patients in different comparison groups by daily consumption of tobacco

Age	Sudden deaths			Non sudden deaths			Survivors			All		
	Total	Pipe	Cigarettes	Total	Pipe	Cigarettes	Total	Pipe	Cigarettes	Total	Pipe	Cigarettes
		or	1 14 15-24 25-		or	1 14 15-24 25-		or	1 14 15-24 25-		or	1 14 15-24 25-
		cigar			cigar			cigar			cigar	
Males												
40-49	4	—	4 10 12	3	—	7 8 8	117	10	17 57 23	169	10	28 75 55
50-59	43	2	8 15 16	36	—	9 1 8	132	6	50 65 31	234	8	87 102 57
60-69	34	—	14 11 9	37	2	11 11 11	6	3	27 22 14	149	6	22 56 34
Total	103	2	26 36 39	69	2	27 42 27	245	18	64 184 78	552	23	148 225 146
Per cent	2	2	25 35 38	3	3	25 42 28	3	6	27 45 23	4	4	37 45 26
Unknown	—	—	—	1	—	—	—	—	—	—	—	—
$\chi^2=13.15$												
Females												
40-49	3	—	1 1 1	2	—	2 1 1	14	—	6 8 8	20	—	10 9 1
50-59	8	—	2 1 4	8	1	2 2 1	29	—	23 13 4	56	1	30 16 9
60-69	3	—	5 1 1	6	—	4 1 1	21	—	16 8 8	36	—	24 10 1
Total	15	—	8 1 5	16	1	11 4 2	76	—	45 29 4	122	1	64 35 11
Per cent	—	—	60 7 22	6	6	61 22 11	—	—	58 36 5	—	1	58 32 10
Unknown	1	—	—	—	—	—	—	—	—	1	—	—
$\chi^2=11.54$												

10 cases (8 male 2 female) of unWitnessed death with known smoking history included.

2 Smokers, daily consumption unknown.

In Table 17 the current smokers have been shown according to their daily consumption of tobacco. Pipe and cigar smokers are entered as a separate group cigarette smokers being divided into three classes.

When all patients with AIHD are considered, it appears that male smokers in general consumed more cigarettes per day than females 76 per cent of all male smokers, against only 10 per cent of all female smokers, smoked more than 25 cigarettes daily and were accordingly classified as heavy smokers. The number of pipe or cigar smokers was small, and there were actually none among the females. The frequency of male heavy smokers was somewhat higher in the youngest age group, whereas in females no correlation with age could be noted.

When the different groups are compared, the most marked difference is the accumulation of heavy smokers in the SCD group in males they account for 56 per cent of all smokers in this group, as opposed to 28 and 23 per cent in the NSCD and SURV group respectively. The corresponding figures for females were 33, 11 and 5 per cent, respectively. The differences in both sexes were almost significant.

Fig. 14 presents the distributions of heavy smokers in the three comparison groups. A striking accumulation is seen in the SCD group and the ratio is also higher than 1 in the NSCD group. The same trend is noted among females, with even more marked differences. The observed-to-expected ratio in the SCD group was no less than 4.25

Comments

During the last decade, the relationship between smoking habit and development of IHD has become well established (U.S. Public Health Service, Public Health Service publication, No. 1103, 1965 No. 1698, 1967 and No. 1696-2, Suppl. 1969 Seltzer 1968, Stamler 1968, Doyle 1969 Seltzer 1970)

In the present study smoking in itself does not seem to influence the course of an AIHD attack. With both sexes the frequency of smoking showed a rather uniform distribution between those who survived their attack and those who succumbed to it. On the whole the prevalence of smoking seems to be very high and higher than in corresponding age groups in the general population of Finland (Kaitaranta et al. 1972)

On the other hand, the accumulation of heavy smokers in the SCD group is seen in both sexes, consistent with previous studies.

Summary

Analysis of the smoking habits of patients with AIHD reveals a high prevalence of cigarette smoking especially in the youngest age group. The frequency of smoking in itself is greatly similar in all comparison groups. Heavy smoking, however seems to be related to sudden death in both sexes.

Physical activity

Data concerning physical activity were lacking in a higher percentage than with regard to most other details of the history. This is because only close relatives or other

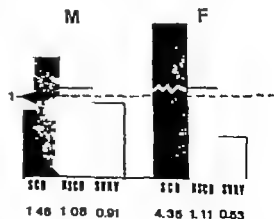


Fig. 14. Preponderance of heavy smokers in SCD NSCD and SURV groups, expressed as observed-to-expected ratios.

Table 12 Distribution of cutaneous, heart disease, patient, non-different comparison groups by physical activity connected with job

Age	Sudden de ths					Non-sudden deaths					Survivors					All					
	Total Data					Total Data					Total Data					Total Data					
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	
40-49	44	79	8	10	8	31	77	7	6	0	150	132	17	46	36	236	192	30	64	51	47
50-59	1	57	18	21	8	11	18	37	19	16	11	239	215	18	78	49	50	397	337	76	72
60-65	53	38	5	14	11	6	72	37	32	16	3	149	133	47	24	23	320	38	1	54	38
Total	200	144	46	45	25	176	141	58	55	25	538	482	122	148	107	955	767	232	324	145	158
Per cent	32	23	8	7	4	27	22	10	9	4	88	79	20	24	17	158	100	39	50	24	25
Male																					
40-49	4	3	1	—	—	2	4	4	3	1	1	—	—	—	—	—	—	—	—	—	—
50-59	16	15	5	2	2	5	10	15	6	3	4	—	—	—	—	—	—	—	—	—	—
60-65	16	14	4	2	7	1	41	34	19	8	7	—	—	—	—	—	—	—	—	—	—
Total	39	32	10	3	9	8	64	53	29	12	12	—	—	—	—	—	—	—	—	—	—
Per cent	31	23	16	7	25	23	33	33	33	33	33	0	0	0	0	0	0	0	0	0	0
Female																					
40-49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
50-59	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
60-65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Per cent	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

5 cases (38 male 13 female) of unwillen death included. R = Retired person.

persons very well familiar with the patients life and habits were able to furnish information reliable enough to enable the physical activity group to be estimated.

Difficulties were caused by the high percentage of retired persons. In the analysis of physical activity linked with occupation, a separate group was formed of these, which was regarded as the group with the lowest activity

Age adjustment was again applied, in view of the close correlation assumed to exist between age and physical activity

The criteria for the different activity groups were fixed in advance. It was noted during the study that a great percentage of patients accumulated in the least active group, and only few persons could be assigned to the most active group in particular in the study of leisure time activity. The original classification was maintained despite this distorted distribution.

Results

Table 18 presents the job-linked physical activity in the comparison groups. Retired persons (R) are shown as a separate zero-group before the least active group I. A considerable percentage of all patients, 30 per cent of males and 37 per cent of females, were retired. This proportion increases strongly with age, of course.

20 per cent of all males belonged to the most active group III. The relative proportion of this most active group was higher than average 22 per cent, in the SURV group. In both other groups, SCD and NSCD it was below average 17 and 18 per cent, respectively. If, on the contrary the retired persons and activity group I are combined to constitute one least active group 61 per cent of all males belong to this combined group. It constitutes 58 per cent of the SURV group, as opposed to 61 and 68 per cent of the SCD and NSCD groups respectively.

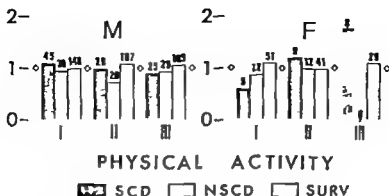


Fig. 15 Preponderance of persons belonging to SCD, NSCD and SURV groups, expressed as age-adjusted observed-to-expected ratios, in different classes of physical activity at work (I + III) Figures over each column. Number of observed cases.

The differences between the comparison groups were statistically almost significant, but this was mainly due to the different numbers of retired persons in different groups.

When females are considered, somewhat greater differences appear to exist between the comparison groups than among males. The frequency of the most active group III is highest in the SCD group (25 per cent) while the NSCD group contained no females at all belonging to this activity group. If, again, the retired persons and activity group I are combined, 63 per cent of all females belong to this least active group. It constitutes only 47 per cent of the SCD group as opposed to 78 per cent in the NSCD group and 81 per cent in the SURV group. The differences between the comparison groups were significant.

Fig. 16 presents the comparison groups as age-adjusted observed-to-expected ratios in three classes of job-connected physical activity. Retired persons are excluded. Among males it can be noticed that the SCD cases are represented in excess of expectation in the least active group I. In the most active group III, the contributions of the comparison groups increase from that of SCD to NSCD and to the SURV group

Among females an entirely different picture is seen. The least active group I displays uniform increase of contribution in the order SCD—NSCD—SURV as in the male activity group III. In both higher activity groups the proportion of sudden deaths is higher than expected. The differences of the ratios were statistically non-significant in both sexes after age adjustment.

The physical activity connected with leisure time is presented in Table 19. A strong accumulation in the least active groups can be seen this is particularly notable in the females. Only a few cases were assigned to the activity group III.

Among males, the prevalence distribution appears to be fairly uniform in the comparison groups. When the females are considered, somewhat greater differences seem to be present. This concerns in particular the SURV group on one hand and SCD and NSCD groups on the other. The frequency of the least active females was higher among those females who died than among those who survived. However these differences are non-significant owing to small numbers.

Fig. 18 again presents the age-adjusted observed-to-expected ratios in the classes of leisure-time physical activity. It must be realized that the most active group III contains very few cases and should be inter-

Table 18 Distribution of acute ischaemic heart disease patients by physical activity connected with leisure time									
Age	Sudden death			Non sudden death			Survivors		
	Total Data	I	II	Total Data	I	II	Total Data	I	II
—19	44	2	14	31	4	13	150	123	63
20-39	51	8	34	73	31	31	228	217	83
40-59	81	14	4	7	34	30	149	129	63
60-69	100	14	83	16	132	74	826	48	251
70-79	53	44	3	38	4	2	46	40	3
80-89	—	—	—	—	—	—	—	—	—
90-99	—	—	—	—	—	—	—	—	—
Total	319	31	177	241	173	164	2114	1773	588
Male									
—19	44	2	14	31	4	13	150	123	63
20-39	51	8	34	73	31	31	228	217	83
40-59	81	14	4	7	34	30	149	129	63
60-69	100	14	83	16	132	74	826	48	251
70-79	53	44	3	38	4	2	46	40	3
80-89	—	—	—	—	—	—	—	—	—
90-99	—	—	—	—	—	—	—	—	—
Total	319	31	177	241	173	164	2114	1773	588
Female									
—19	—	—	—	—	—	—	—	—	—
20-39	—	—	—	—	—	—	—	—	—
40-59	—	—	—	—	—	—	—	—	—
60-69	—	—	—	—	—	—	—	—	—
70-79	—	—	—	—	—	—	—	—	—
80-89	—	—	—	—	—	—	—	—	—
90-99	—	—	—	—	—	—	—	—	—
Total	—	—	—	—	—	—	—	—	—

preted with great reserve. Among males in the activity groups I and II, all three comparison groups appear to be closely equal. However the SURV group is slightly under-represented compared to the expected level in the least active group and over-represented in the more active group III and also in the activity group III. SCD and NSCD both surpass the expected level in the least active group and fall below it in the activity group II.

Among females the ratios in the least active group I decrease uniformly in the order SCD — NSCD — SURV. The reverse is seen in the more active group II. Only three females belonged to the most active group III and they all survived.

The age-adjusted ratios given did not reach a significant level, however either in males or in females.

Comments

Many authors have pointed out that studies of physical activity have so far been hampered by obvious shortcomings and sources of error which are difficult to avoid (Taylor 1967, Altekrose 1968, Paul 1969, Kannel 1970). The methodological difficulties were also clearly perceived in the present study. The validity of the questionnaire remains to be established although an attempt was made to test it, as presented in Chapter III.

The study population represents the whole spectrum of AIHD in the community and a considerable proportion of all patients were previously retired. On the other hand, this implies that the subjects' previous health varied greatly and a substantial proportion of them have had to restrict their physical activity due to illness. Cause and effect become mixed.

Occupations which necessitate at least moderate physical activity are becoming fewer all the time (Morris and Gardner 1969). In fact the occupational differences in job-connected physical activity are already now

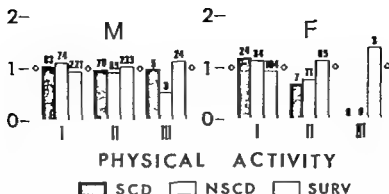


Fig. 16. Preponderance of persons belonging to SCD, NSCD and SURV groups, expressed as age-adjusted observed-to-expected ratios, in different classes of physical activity during leisure time (I to III). Figures over each column: number of observed cases.

so small under urban conditions that highly accurate methods would be needed to show them up. At the same time, the role of leisure time activity is probably increasing in importance. In a middle-aged or older urban population, however, habitual physical activity seems to be very infrequent or even spurious.

Finally it must be emphasized that although physical activity may have an independent effect of its own on mortality in IHD its influence is without doubt closely related to many other risk factors. In the foregoing, an attempt was made to eliminate one of them, namely age. Many others, though, remain.

Considering all the reservations made in the foregoing, it is not surprising that only a very slight association between physical activity and the prognosis of an AIHD attack was found. After age adjustment no significant differences remained between the comparison groups.

However the results presented above disclose a slight tendency at least among males, of increasing physical activity being associated with survival more often than with death, though not sudden death in particular. The most prominent finding as regards females was the inverse association found for physical activity on the job on one hand and for leisure time activity on the other

The method used does not allow for any combination of both effects, even though this would be highly desirable. One explanation of the contradictory results among females is certainly furnished by the difficulty of drawing the line between job-connected and leisure time-connected physical activity especially among housewives.

Summary

The job-connected and leisure time-connected physical activity were both determined for patients with AIHD. A slight trend was found among males towards more frequent association of survival with high physical activity and correspondingly of death with physical inactivity rather than vice versa. There was no clear association with sudden death in particular. The same was observed as regards the physical activity of leisure time among females, but consideration of their physical activity connected with the job revealed an inverse association. This may be due to the indefinite demarcation of job and leisure time especially among housewives.

Many methodological difficulties are encountered in a study of the present kind in attempts to determine the potential association of physical activity with the prognosis of an AIHD attack.

Striped platy has been considered a model organism in animal behavior research and the model organism for the study of the effects of environmental factors on behavior.

Male										Female									
Continuous variable										Continuous variable									
None one more than one										None one more than one									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Smoker Ex-smoker Non-smoker										Smoker Ex-smoker Non-smoker									
0 1-4 5-14 15-25 25--										0 1-4 5-14 15-25 25--									
Continuous variable										Continuous variable									
I, II III (Increasing level of physical activity from I to III)										I, II III (Increasing level of physical activity from I to III)									
I, II III (I, II III (
I II III IV (Decreasing social level from I to IV)										I II III IV (Decreasing social level from I to IV)									

Male										Female									
Continuous variable										Continuous variable									
None one more than one										None one more than one									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Smoker Ex-smoker Non-smoker										Smoker Ex-smoker Non-smoker									
0 1-4 5-14 15-25 25--										0 1-4 5-14 15-25 25--									
Continuous variable										Continuous variable									
I, II III (Increasing level of physical activity from I to III)										I, II III (Increasing level of physical activity from I to III)									
I, II III (I, II III (
I II III IV (Decreasing social level from I to IV)										I II III IV (Decreasing social level from I to IV)									

Male										Female									
Continuous variable										Continuous variable									
None one more than one										None one more than one									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Smoker Ex-smoker Non-smoker										Smoker Ex-smoker Non-smoker									
0 1-4 5-14 15-25 25--										0 1-4 5-14 15-25 25--									
Continuous variable										Continuous variable									
I, II III (Increasing level of physical activity from I to III)										I, II III (Increasing level of physical activity from I to III)									
I, II III (I, II III (
I II III IV (Decreasing social level from I to IV)										I II III IV (Decreasing social level from I to IV)									

Male										Female									
Continuous variable										Continuous variable									
None one more than one										None one more than one									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Yes, no										Yes, no									
Smoker Ex-smoker Non-smoker										Smoker Ex-smoker Non-smoker									
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6. MULTIFACTORIAL ANALYSIS OF THE FACTORS RELATED TO THE GROUPS OF SUDDEN DEATHS, NON-SUDDEN DEATHS AND SURVIVORS

In the foregoing, several characteristics and previous diseases of the patients with acute ischaemic heart disease were separately considered in the comparison groups, SCD, NSCD and SURV. On the other hand, it is well known that the risk factors of IHD are mutually connected in several ways and by no means exert their influence independent of each other.

One of the aims of the present study as presented in Chapter I was to test whether it might be possible to differentiate the SCD group from the other comparison groups (NSCD and SURV) on the basis of known risk factors, previous diseases or other characteristics. Therefore, a method was applied which enabled all chosen factors to be taken into account simultaneously. The method used was multiple discriminant analysis (Sarna 1970, Cooley and Lohnes 1971).

Results

Thirteen variables were chosen to represent established and suspected risk factors of IHD

as well as symptomatic IHD in itself. The variables and their classification are presented in Table 20. If the value of one or two variables was unknown, the mean of the variable or variables in question was substituted for the data lacking. Cases lacking in information regarding more than two variables were excluded from the analysis.

In order to test for differences between the prognostic groups, all variables were tested both separately and in aggregate. The F test was used in the first instance and Wilks' lambda test in the second.

Table 21 shows the mean value for each variable in the three comparison groups, as well as the corresponding F values and their degrees of freedom. It is seen that most of the F values are relatively low implying poor discriminative power of these variables. The value found for Wilks' lambda was 0.911057 and the value for its F approximation was 3.836, with 26 and 2092 degrees of freedom. This reveals that the total discrimination power of the chosen variables was also low.

Table 22 presents the thirteen chosen variables in descending order of their reducing effect on Wilks' lambda, after elimination of the effect of intercorrelations. The

Table 22. Multiple discriminant analysis variables, listed in descending order of their reducing effect on Wilks' lambda

In brackets, ordinal number of each variable (see Table 20).

Variable		Lambda	F test for additional information
1. Stroke	(8)	0.965	F (2, 2140) = 42.4
2. Body mass index	(10)	0.972	F (4, 2124) = 1.5
3. Diabetes mellitus	(6)	0.962	F (8, 2122) = 9.5
4. Sex	(1)	0.934	F (8, 2122) = 8.2
5. Age	(3)	0.944	F (10, 2124) = 2.8
6. Daily consumption of cigarettes	(9)	0.936	F (12, 2120) = 2.4
7. Myocardial infarction	(3)	0.928	F (14, 2116) = 1.4
8. Physical activity connected with leisure time	(12)	0.922	F (16, 2112) = 1.1
9. Hypertension	(7)	0.917	F (18, 2108) = 0.4
10. Physical activity connected with work	(11)	0.914	F (20, 2104) = 0.2
11. Smoking habit	(5)	0.912	F (22, 2100) = 0.03
12. Social class	(13)	0.911	F (24, 2096) = 0.005
13. Angina pectoris	(4)	0.911	F (26, 2092) = 0.000

Table 21. Correlation matrix of the multiple discriminant analysis variables. Entries made only where statistical significance exists

	$p < 0.05$ $** p < 0.01$ $*** p < 0.001$												
Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Sex													
2 Age	***												
3 Myocardial infarction													
4 Angina pectoris		**											
5 Stroke		**	**										
6 Diabetes mellitus													
7 Hypertension		**			**								
8 Smoking habit	**	**		**			**						
9 Daily consumption of cigarettes			**	**				**					
10 Body mass index			**										
11 Physical activity connected with work		**											
12 Physical activity connected with leisure time													
13 Social class				**						**			

corresponding lambda value and F approximation are given for each variable. The F test was used in this connection to test the additional information which each variable contributes to total discriminant function. Because of the mutual correlations of various variables, the order is not the same as indicated by the F values of the same variables in Table 21. For instance age, which has the second highest F value ranges only fifth in this table.

Table 23 presents the correlation matrix between the variables. Only the significances of the correlations are given.

In order to test in greater detail the two discriminant functions the eigenvalues for both were determined (Table 4). It can be seen that 77 per cent of the total discriminant power falls to the share of the first discriminant function, which mainly fulfills the task of separating the survivors from those who died. Only 3 per cent of the total remained for the second dis-

criminant function, which mainly separates the two death groups, that of sudden and that of non-sudden death.

Table 25 presents the correlations of the variables with the two discriminant functions. The quantitative effect of each variable can be seen. Table 26 shows the mean values for the comparison groups in the two sub-dimensions formed by the two discriminant functions. Finally in Fig 17 the means of each group are presented in the differential space. Tables 25 and 26 together give an overall picture of the differences between the comparison groups.

Table 4. Eigenvalues of the two discriminant functions

Discriminant function	1	0.07438	77
Discriminant function	2	0.01145	2.3
Wilks lambda	0.91103		
F (16, 107)	3.836		

Table 25. Correlations between the multiple discriminant analysis variables and the two discriminant functions.

Variable	Discriminant function	
	1	2
1. Sex	0.199	0.495
2. Age	-0.428	-0.072
3. Myocardial infarction	-0.410	0.181
4. Angina pectoris	0.242	-0.009
5. Stroke	0.471	-0.054
6. Diabetes mellitus	0.319	-0.400
7. Hypertension	0.168	0.272
8. Smoking habit	-0.006	0.086
9. Daily consumption of cigarettes	-0.163	-0.366
10. Body mass index	0.411	-0.206
11. Physical activity connected with work	0.203	-0.262
12. Physical activity connected with leisure time	0.340	-0.125
13. Social class	0.078	-0.134

It can be found that the male sex exerts a weak effect towards fatal outcome and, in the second discriminant function, a strong influence towards sudden death. Increasing age adds markedly to the chance of dying, but it has only a minimal effect on the mode of death. A positive history of myocardial infarction also increases the possibility of death and has a slight influence favouring non-sudden death. A history of angina pectoris has a slight influence to death, but it is almost without effect on the mode of death. A history of stroke strongly increases mortality but it, too, is devoid of effect on the mode of death.

Table 26. Mean values for the comparison groups in two subdimensions formed by the two discriminant functions.

Discriminant function	Sudden deaths		Non-sudden deaths		Survivors	
	1	2	1	2	1	2
Mean	1.894	-7.171	1.724	-6.690	2.370	-6.878
S.D.	1.110	0.9682	1.058	1.139	0.9495	0.9421

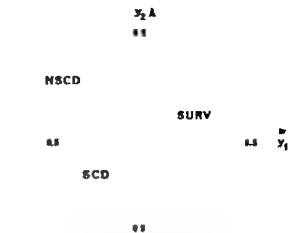


Fig. 17 The comparison groups, SCD, NSCD and SURV plotted in differential space. The total mean vector (2.172, -6.800) was linearly transformed to zero.

Diabetes mellitus favours death and non sudden death, while hypertension promotes death, but primarily sudden death, although this latter influence is relatively weak. Whether any given person is a smoker ex smoker or non-smoker has almost no effect on the prognosis of an AIHD attack. On the contrary if current smokers alone are considered, the heavy smokers have a slight tendency to die and if so, they appear to die suddenly. Relative weight exerts a relatively strong influence in the direction of survival and, on the other hand, in the direction of sudden death. The increasing physical activity during work and leisure time both have a slight effect in favour of survival and, in the second discriminant function, a weak effect in favour of sudden death. Finally social class has virtually no effect at all in either one of the discriminant functions.

Comments

A multifactorial analysis conveys an overall picture of the significance of the chosen variables which is different, to a certain degree, from that obtained by the usual observation method. All relevant variables may be considered in a quantitative manner both individually and as intercorrelated variables. Those variables yielding no additional information can be excluded. The direction of the effect of each variable is discernible even if it is minimal. The method used is accurate in contrast to the information gathered, which unfortunately was too vague and unreliable to allow for efficient discrimination.

Unavoidable drawbacks are associated with a study of the present kind, in which information is gathered in a retrospective manner. The history of previous diseases and habits is not very reliable and the validity of information varies in different comparison groups. Most of the survivors } were personally interviewed, as were some of the patients in the non-sudden death group. Case records from the hospital, at least, were almost invariably available. In contrast, the history of persons who died suddenly was almost exclusively based on interviews of their spouses or other relatives. Owing to the small differences between the comparison groups the significance of information lacking became very marked. This was clearly experienced in the attempts to find the most appropriate manner of dealing with unknown data.

Retrospective studies also preclude quantitative investigation of previous diseases in that often only a dichotomic classification is feasible. Compared with a prospective study, this is an obvious drawback in the case of variables such as hypertension. The importance is further accentuated if one considers that a substantial percentage of persons are unaware of the hypertension on

Only two variables could be analyzed as continuous variables, namely age and relative weight. As for the other variables merely an arbitrary classification could be set up. The classification of dichotomic variables is rough, but at all events distinct. On the other hand variables such as smoking habit and social class had to be divided into classes without any assurance of their linearity and appropriate scale.

The list of predominance of the variables, presented in Table 22, is surprising to a certain extent. It appeared that the history of stroke had the best discriminant power of all variables chosen, after the intercorrelations between the variables had been eliminated. A positive history of stroke is strongly associated with death, but the mode of death was more often that of delayed death. In this connection a previous stroke can be considered an expression of advanced arteriosclerosis. It must be emphasized, however, that the discriminant power of all chosen variables was relatively low and, therefore the order of the variables is of less importance.

The prevalence of different previous diseases and risk factors in the comparison groups has been considered separately before. It was clearly evident that the differences were very small indeed on the whole. Therefore it is not unexpected that multiple discriminant analysis was also unable to separate the groups very well. It appears that it is hardly possible, on the basis of the patient's history only to predict in advance which persons are going to die when an AMHD attack occurs and even less which ones will die suddenly. Prospective studies are capable of producing more reliable information like the results of clinical examination, laboratory tests and electrocardiograms, for instance. It is, therefore, believed that the outlook as regards predicting the fate of individual persons may also be rather different in this connection.

Summary

A multiple discriminant analysis was applied in order to test the possibility of separating the three comparison groups, i.e. the SCD NSCD and SURV groups, on the basis of the history of previous diseases and other risk factors. Thirteen variables were chosen for the analysis. Unfortunately the discrimination power of the variables was relatively low 77.7 per cent of the total discriminant function falls to the first discriminant function which separates the survivors from those who died. Only 22.3 per cent of the total remained for the second discriminant function which separates the SCD and NSCD groups.

Factors which add to the chance of dying are the male sex, increasing age, a positive history of myocardial infarction, angina pectoris, stroke, hypertension or diabetes mellitus, and low physical activity. In the second discriminant function especially male sex had a strong influence in the direction of sudden death. The heavy smokers have also a tendency to die suddenly. Increasing relative weight has an influence in the direction of survival and, on the other hand, in the direction of sudden death. Social class had no effect at all.

The available, comparatively unreliable information on the above-mentioned factors seemed to offer no opportunity for pointing out in advance, with sufficient accuracy the persons at the highest level of risk of dying suddenly.

7 PREMONITORY SYMPTOMS

A query was made concerning premonitory symptoms during the last four weeks before the AIHD attack in all cases of the present study. The following symptoms in particular were inquired after: angina pectoris of recent onset; a change in pattern of previous angina pectoris, discomfort in the chest, heaviness of the arms, unusual dys-

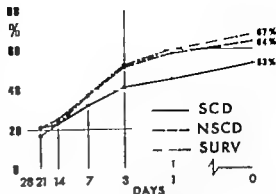


Fig 18. Cumulative graphs showing occurrence of premonitory symptoms during 28 days prior to acute ischaemic heart disease attack in the SCD NSCD and SURV groups.

pnoea, unusual tiredness or first occurrence of palpitation. Only those symptoms which really had appeared or had changed their character within this period were accepted as premonitory symptoms.

Results

A high frequency of prodromal symptoms was reported in all three comparison groups. The frequency was highest in the SURV group (67 per cent) followed by the NSCD group (64 per cent) and lowest in the SCD group (53 per cent). The given percentages refer to cases in which the history of symptoms during the preceding month was available and in which one or several of the symptoms were reported. The differences were statistically almost significant.

Fig 18 shows the times at which premonitory symptoms occurred in the comparison groups, in the form of cumulative prevalence graphs. All three graphs run closely parallel with each other although that of the SCD group stays somewhat lower at the end. According to the figure, the frequency of prodromal symptoms appearing during the fourth week before the attack is fairly high in all groups during the two consecutive weeks it remains lower and an abrupt rise once more occurs during the last week.

Fig. 19 presents the frequency of premonitory symptoms in all three comparison groups. The percentages given refer to the total number of patients in each subgroup. The frequency of those symptoms which were considered most important, namely angina pectoris and unusual dyspnoea, is also presented. It can be seen that the frequency of reported angina pectoris was definitely higher in the SURV group (33 per cent) than in the NSCD (22 per cent) and SCD groups (18 per cent). These differences were statistically highly significant. The contribution of unusual dyspnoea was less and differences between the comparison groups were also small.

The frequency of those persons who were reportedly without any prodromal symptoms was on the same level in all subgroups. In contrast, the amount of unknown data was substantially higher in both groups of dead subjects, as opposed to the SURV group.

Table 27 states the frequency of visits to a doctor over the same period of four weeks preceding the acute attack. It has been found that more than half of all patients in the SCD and NSCD groups had seen a doctor during this period. Only minimal differences exist between these two groups. Visits to a doctor were far more infrequent in the SURV group. Compared with the SCD group, the difference was highly significant for males and almost significant for females. At the bottom of Table 27 the relative number of those visits has been stated which were made on account of premonitory symptoms. It can be seen that in the SURV group consulting a doctor for premonitory symptoms was relatively more common especially among males, differing almost significantly from the SCD group.

Comments

Herrick (191) had already noticed that a symptomatic phase often preceded a myocardial infarction. In 1915 F. J. as well as

Symptoms	All Cases	SCD	NSCD	SURV	
Not Known	1215	229	240	736	100
	88	34	33	14	%
None	464			239	
		89	75		
Miscellaneous	287			177	59
			34		
Dyspnoea*	82	82		59	
			30		
Angina Pectoris	111	18	22	133	
		21	33		

* Those with Angina Pectoris included

Fig. 19 Prevalence of premonitory symptoms in the SCD, NSCD and SURV groups.

Sampson and Eliaser estimated the frequency of premonitory symptoms before a heart attack, which they found to be 50 and 48 per cent respectively. Recent studies of hospital patients with a myocardial infarction have demonstrated even higher rates. Solomon et al. (1969) 63%, Stowers and Short (1970) 68%, and Hochberg (1971) 84%.

It has been more difficult to gain reliable information on premonitory symptoms of sudden death. Adelson and Hoffman (1961) in a study consisting of 500 consecutive sudden deaths defined as occurring within two hours, noticed prodromal symptoms in 49 per cent of cases. Bainton and Peterson (1963) reported that prodromal symptoms occurred in 59 per cent of fatal cases of AIHD. In a Danish study consisting of autopsied sudden coronary deaths at a young age 45 per cent of cases presented prodromal symptoms more than 24 hours prior to death (Hansen 1968). In Oxford, England, Kinlen (1959) noted a recent onset or change in pattern of pain in the chest or epigastrium during the last month before the heart attack, in 49 per cent of patients with a

Table III Visits to a doctor of acute ischaemic heart disease patients during four weeks preceding the attack.

	Sudden deaths				Non-sudden deaths				Survivors			
	Males		Females		Males		Females		Males		Females	
	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent
Total	200		39		176		64		538		198	
Data available	170		32		153		56		529		194	
Time prior to attack												
—1 week	39	23	10	31	46	30	16	29	76	13	39	20
1—3 weeks	22	13	4	13	13	8	3	5	39	7	20	10
3—4 weeks	29	17	2	6	28	18	11	30	58	11	25	13
Total visits	90	53	16	50	86	56	30	54	175	33	84	43
Visits caused by premoni- tory symptoms	30	33	7	44	39	45	10	33	82	47	28	43

myocardial infarction, and in 33 per cent of patients who died suddenly.

The frequency of all reported symptoms in the present study seems to be on the same level as in the above-mentioned studies. It is possible that the lower frequency noted in the SCD group compared to the NSCD and SURV groups, is merely due to the fact that the history of patients who die suddenly is usually less accurately known than that of patients in the other groups. This perhaps concerns rather more details such as premonitory symptoms than, for example, the history of previous diseases, of which the relatives probably are more often aware.

An obvious shortcoming, and in fact serious limitation, of all above-mentioned studies, including the present one, is their retrospective nature. Preliminary results from a prospective study on unstable angina have lately been reported from Edinburgh, and they convey a somewhat different picture (Fulton et al. 1972). Although registered unstable angina progressed to a myocardial infarction in 14 per cent of cases, sudden death was an infrequent sequela.

A change in angina pectoris is usually considered the most important symptom in-

dicating a potential risk, but its frequency in the present study is fairly low in the SCD group. On the other hand, it is impossible to presume that all patients who complained of indefinite symptoms like tiredness could have been taken under close medical surveillance. Therefore it seems unlikely that persons experiencing SCD could be separated from persons in whom the course of an AIHD attack is less severe, on the basis of prodromal symptoms.

More than half of all patients who died in an AIHD attack had visited a doctor during the preceding month, and more than one-third of these visits were caused by prodromal symptoms. The frequency seems surprisingly high, but similar frequencies have also been reported earlier: 40 per cent of victims from sudden death in Edinburgh (Fulton et al. 1972), and 50 per cent in Oxford (Kinlen 1969) had seen their doctor within the preceding month. In Oxford 25 per cent, and in Baltimore (Kuller et al. 1966a) 24 per cent, of patients who died suddenly had visited a doctor during the preceding week. The corresponding figure for the last week was 24 per cent in the present study.

It is conceivable that a high frequency of visits to a doctor might offer an opportunity to take the patients at risk under medical control at the most appropriate phase. On the other hand, the greater part of the visits were for reasons other than premonitory symptoms of an AIHD attack. The character of the premonitory symptoms is mostly so indefinite that it is hardly to be expected that their serious nature would be correctly interpreted by the patient or his doctor. It is however worth while to note that roughly 18 per cent of all patients who died suddenly paid enough attention to their premonitory symptoms to make them see their doctor for them.

Summary

53 per cent of patients in the SCD group were reported to have had some premonitory symptoms during the preceding four weeks before the attack, and 18 per cent experienced a change in their angina pectoris symptoms. The corresponding figures were somewhat higher in the other groups.

More than a half of the patients who succumbed to sudden death saw their doctor during the preceding four weeks, and 24 per cent during the last week. More than one-third of the visits were caused by premonitory symptoms.

8 ATTENDANT CIRCUMSTANCES OF THE ACUTE ATTACK

Accurate knowledge of the circumstances in which the sudden coronary fatalities occur also seems necessary before any control programme can be planned. The place and time of occurrence the activity at onset of symptoms as well as the steps taken to obtain medical help were considered essential points which require clarification. Since in general the fatal attacks were witnessed, this information was in fact obtained in a great majority of cases.

Table 28 Distribution of sudden death cases by sex and time of day of onset of attack.

Time of day hrs	Males	Females	Total
00-06	28	6	44
06-12	53	10	63
12-18	55	9	64
18-24	64	14	78
Total	200	39	239
	$\chi^2=3.68$ N.S.D. (Males)	$\chi^2=3.36$ N.S.D. (Females)	

Results

Table 28 presents the time of occurrence of the fatal attack in sudden death cases. The attacks seem to occur quite evenly throughout the 24-hour day except during the first six hours, from 12 p.m. to 6 a.m., when definitely fewer attacks occurred than were expected.

Table 29 shows the activity in which the patients were engaged when their acute attack occurred. It can be seen that the activity was rather closely similar in all three comparison groups. The majority of the attacks occurred at rest. Strenuous activity was only rarely reported more often among males (in 6 per cent) than among females (in 2.5 per cent). Eight entries of strenuous activities in the SCD group consist of four patients engaged in sport, three who were mounting stairs and one who was carrying a heavy object when death occurred.

In addition to the activity at the exact moment of occurrence of the acute attack symptoms, the relatives were further asked concerning any extraordinary stress experienced by the victim immediately before the attack. Various kinds of extra strain were reported. The reports in the SCD group include 2 cases of exceptional physical stress (9 per cent of all SCD cases). The reported activities mainly consisted of sport activities or of heavy household work, such as shovelling snow etc.

Table 29. Distribution of acute ischaemic heart disease patients in different comparison groups by activity at onset of attack.

Activity	Sudden deaths		Non-sudden deaths		Survivors		Total No.	Per cent
	No.	Per cent	No.	Per cent	No.	Per cent		
Males								
Rest	118	63.4	90	62.9	295	58.1	516	60.6
Work	61	32.8	44	30.8	18	35.0	223	33.5
Strenuous activity	7	3.7	9	6.3	35	6.9	51	6.0
Total, activity known	186	100.0	143	100.0	508	100.0	832	100.0
Activity unknown	14		33		30		101	
Female								
Rest	20	55.6	33	63.5	116	63.4	178	62.9
Work	15	41.8	18	34.6	62	33.9	96	34.8
Strenuous activity	1	2.8	1	1.9	5	2.7	7	2.5
Total, activity known	36	100.0	52	100.0	183	100.0	278	100.0
Activity unknown	3		12		15		28	

Twelve cases of exceptional mental stress were reported, in two cases a severe quarrel had shortly preceded the patient's death. Five patients bathed in sauna shortly before the attack. In seven cases, the use of alcohol preceded death.

Table 30 presents the places of onset of symptoms in the three comparison groups. It can be seen that the majority of attacks occurred at home, more frequently among females (80 per cent) than among males (58 per cent), and more frequently among survi-

Table 30. Distribution of acute ischaemic heart disease patients in different comparison groups by place of onset of attack.

Place of onset	Sudden deaths		Non-sudden deaths		Survivors		Total No.	Per cent
	No.	Per cent	No.	Per cent	No.	Per cent		
Male								
Work place	13	6	19	11	66	12	97	10
Home	95	48	76	46	341	64	536	58
Other place	67	34	33	20	93	17	199	21
Hospital	25	12	12	7	14	3	51	5
Onset indefinite	—	—	27	16	20	4	49	5
Total known	200	100	166	100	534	100	932	100
Unknown	—		10		4		21	
Female								
Work place	1	3	2	3	8	5	17	4
Home	22	36	40	63	162	72	212	69
Other place	13	33	8	8	25	13	43	18
Hospital	3	8	11	18	19	5	25	8
Onset indefinite	—	—	4	6	11	6	15	5
Total known	39	100	62	100	197	100	307	100
Unknown	—		2		1		7	

Table 31. Distribution of acute ischaemic heart disease patients in different comparison groups by place of death.

Place of death	Sudden deaths		Unwitnessed deaths		Non-sudden deaths		Total	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Males								
Work place	12	6	—	—	—	—	12	3
Home	85	43	26	68	22	13	133	31
Other place	64	32	12	31	18	9	90	22
Hospital	25	13	1	2	127	73	153	37
During transport	11	6	—	—	4	2	15	4
Outpatient department	3	2	—	—	8	3	9	2
Total known	200	100	39	100	174	100	413	100
Unknown	—	—	—	—	2	—	2	—
Females								
Work place	1	3	—	—	—	—	1	1
Home	21	54	9	75	4	6	34	30
Other place	12	31	2	17	1	—	13	13
Hospital	3	8	1	8	56	88	60	52
During transport	—	—	—	—	2	3	4	3
Outpatient department	—	—	—	—	1	2	1	1
Total known	39	100	12	100	64	100	115	100
Unknown	—	—	1	—	—	—	1	—

vers than among those who died. Home was also the commonest place of occurrence in the SCD group although, on the other hand, in this group the category 'other places' was proportionally more strongly represented than in the other two groups. 3 per cent of males and 8 per cent of females AIHD attacks were experienced by persons already hospitalized for various other reasons.

Table 31 similarly shows the places of death. In addition to the SCD and NSCD groups, the unwitnessed deaths were also included in the table. Deaths in transit to hospital and deaths at the outpatient department of a hospital within one hour of arrival, have been distinguished as separate categories because of the practical importance of this information. Both categories remained small: only 4 per cent of all AIHD deaths occurred in transit and 1 per cent immediately after arrival in hospital.

The efforts made to transport to a hospital those patients who ultimately fell into the SCD group have been detailed in Fig 20. The initial grouping of these patients is according to the place of onset of their symptoms. The diagram reveals that a great majority of the patients died at the same

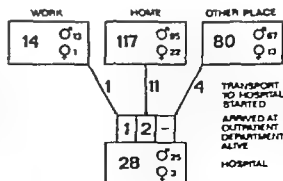


Fig 20 The fate of the SCD cases, presented according to the place of onset of symptoms with particular reference to efforts made to transport the patient to hospital.

place before any transport was available. If patients only were probably still alive when their journey to the hospital commenced. Ultimately only three patients arrived alive at the outpatient department, while the rest had all died in transit.

In only four other cases the ambulance was summoned while the patient was still alive, but it did not reach the patient before he died. In two further cases a physician on duty was called while the patient was still conscious, and he reached the patient prior to death in one of the two cases.

Comments

The exceptionally small number of sudden deaths during the night hours is obviously due to the fact that many such deaths remained unwitnessed and fell into the unwitnessed death category rather than into the SCD group. Therefore, the sudden death cases are not thought to be associated with any particular time of day.

The majority of deaths seem to have occurred while the patient was sleeping or resting, while association of death with strenuous activity was rare. This is in agreement with several previous studies (Rabson and Helpers 1948, Spain and Braden 1957, Weinberg and Helpers 1959, Adelson 1961, Spiekerman et al. 1962, Stevens 1963, Peil and D'Alonzo 1964, Spain 1964, Kuller 1966, Kuller et al. 1966a, Hansen 1968, Wikland 1968, Lindström 1969, Moss et al. 1969, Solomon et al. 1969, Paul and Schatz 1971, Wikland 1971). Differing from the general consensus, Yater et al. (1948) found that acute attacks of young persons occurred more often during heavy physical activity than could be expected.

It is naturally very difficult or even impossible to assess the true frequency of strenuous activity in the population at large. The results, therefore, justify only the conclusion that strenuous activity probably does not significantly influence the course and

termination of an AIHD attack when it occurs. The higher frequency of strenuous activity among males compared with females obviously merely reflects the males more frequent pursuit of such activities. Finally, it must be stated that the strenuous activities reported cannot by any means be considered extraordinarily heavy for a healthy person.

It is possible, on the other hand, that extra strain before the attack could be more important than that undergone at the very moment when the first symptoms of an AIHD attack appear. The consideration may further be extended to comprise not only physical stress but also unusual mental stress. The time interval during which such factors can be assumed to exert an influence is not known.

The frequency of extra strain during the period immediately prior to sudden fatality was higher than at the onset of the attack, but the inquiry also concerned a period of two to three hours. Reported mental stress mainly consisted of various kinds of daily trouble. In two cases a severe quarrel preceded death. The reported sauna episodes and indulgence in alcohol can hardly be considered anything but spurious.

A closer analysis of all reported preceding stresses reveals that the great majority can hardly be considered unusual. It is also probable that the relatives tend to over-emphasize the importance and the degree of preceding strains.

The distribution according to the place of onset of symptoms found here resembles those in previously reported studies. It is only natural that the most common place should be the home since the patient spends the greater part of his time at home especially the females.

Several studies have shown that approximately two thirds of all IHD deaths occur out of the home. Of course this percentage is much higher if only cases coming to the attention of the medical authorities are taken

all aid comes too late and the patient is lost if nothing is done before medical aid is obtained. Four minutes is the critical time interval in cardiac arrest if circulation and respiration is not maintained. In theory it might be possible to teach resuscitation procedures to laymen, in particular to the relatives of patients with known IHD. It has also been suggested that patients who have symptomatic IHD should be provided with self-administered medicines to enable them or their relatives to give them a rhythm-stabilizing injection at once if an attack occurs (Levine 1969). However it is hardly realistic to assume that the measures proposed could significantly decrease the acute mortality of AIHD attacks.

Not even prodromal symptoms seem to distinguish the victims of sudden death from persons with a more favourable course for their attack. It has been proposed that special pre-coronary care stations should be established in order to observe those patients who display alarming symptoms, such as a change in the pattern of angina pectoris (Lown et al 1969a). On the other hand a recent report from Edinburgh (Fulton et al 1977) seems to indicate that unstable angina pectoris is no good predictor of subsequent sudden death.

Although various measures can be discussed speculatively (Sidel et al 1969; Yu 1971), it remains that the possibilities of intervention of any kind either at the onset of the attack or during the prodromal phase seem to be limited in those cases characterized by the most acute course. There

remains the possibility of prevention, but as has been mentioned earlier the immediate risk group cannot be identified with sufficient accuracy. At present, the natural risk group consists of persons who already have symptoms of ischaemic heart disease. Two-thirds of the persons dying suddenly in the present study had suffered from a symptomatic IHD before their death.

All preventive measures, whether primary or secondary which decrease the incidence of AIHD attacks probably also decrease the incidence of sudden coronary death. Unfortunately at present, too little is known concerning the most suitable methods and concerning the effects of any large-scaled programme.

When prevention of sudden coronary death is considered, the basic question is, however how to prevent fatal disturbances of the heart rhythm. Numerous antiarrhythmic medicines are available. Unfortunately none of them seems to be suitable for long term prophylactic use in large populations. A suitable medicine should be both effective and safe. For example, procainamide and quinidine though often effective contain a potential risk of dangerous side-effects. Too little is known in this respect about the beta blocking agents. Quite recently clofibrate has given promising results in decreasing the sudden coronary death rate (Ischaemic Heart Disease: A secondary prevention trial using clofibrate 1971 and Trial of clofibrate in the treatment of ischaemic heart disease 1971).

GENERAL SUMMARY

During one year 1970 1267 cases of acute ischaemic heart disease, 953 males and 314 females, fulfilling the minimum criteria of WHO were registered in the Helsinki population aged 65 years or younger. The case fatality rate was 43.6 per cent for males and 37.1 per cent for females. 239 cases (18.8 per cent of all AIHD cases) represented sudden coronary death cases, defined as deaths occurring within one hour from the onset of symptoms. These cases and their characteristics were the actual object of the present study. Cases with a different outcome, i.e. non-sudden death or survival constituted the comparison groups.

Deaths within one hour constituted a remarkable proportion of all AIHD deaths, 53.1 per cent of all male and 38.5 per cent of all female deaths with known duration of the fatal attack. At the same time these figures reveal a marked sex difference in the course of an AIHD attack, sudden death being far more often the fate of males than of females. The incidence of sudden coronary death increases strongly with age in both sexes, but its relative proportion of all AIHD cases is rather closely similar in different age groups. The incidence seems to be high, although figures of an equivalent level have been reported earlier from other countries having a high IHD mortality in general.

The prevalences of certain demographic characteristics, previous diseases and established risk factors were determined in the comparison groups. The aim of this part of the study was to find those characteristics, if any which might help to identify in

advance those in great risk of dying suddenly.

It was found that the above-mentioned comparison groups were very similar with regard to most of the factors observed. After age adjustment no particular marital status category or social class was significantly associated with any one of the comparison groups, SCD NSCD or SURV. A history of previous myocardial infarction was more often associated with death than with survival of an AIHD attack, but the death was more often delayed than sudden. The prevalence of hypertension was highest in the SCD group and lowest in the SURV group. The highest prevalence of diabetes mellitus was found in the NSCD group. Only 20 per cent of males and 9 per cent of females could be regarded as being free from any clinical cardiovascular disease or diabetes mellitus prior to the attack. These persons had a better prognosis when an AIHD attack occurred. The body mass index was lowest in the NSCD group and on the same level in the other comparison groups, SCD and SURV. The frequency of smoking was very similar in all comparison groups. On the other hand, heavy smoking seemed to be related to sudden death. Physical activity seemed to have a slight influence favouring survival.

An attempt was made to separate the three comparison groups, SCD NSCD and SURV by the aid of multifactorial analysis, i.e. multiple discriminant analysis. Although this method enables all selected factors to be considered simultaneously both together and separately in a quantitative manner the

end result was that on the basis of the information collected it was not possible to predict very well which persons' AIHD attacks would terminate in sudden death.

The analysis of premonitory symptoms revealed that a high percentage of all AIHD patients had complained of some symptoms during the preceding month. In the group of sudden deaths their prevalence was lowest, but the difference was small. The frequency of change in the pattern of angina pectoris, regarded as the only symptom which could be considered specific in some degree was also lowest in the group of sudden deaths.

The place of onset of symptoms was most often the home just as it was in the comparison groups. Most attacks occurred at rest and those in connection with strenuous activity were very rare. The great majority of patients whose ultimate fate was sudden death died at the same place where the attack began and only a few patients could be brought under adequate medical care. This was obviously due to the unfavourable duration of the attack, not to the inadequacy of the emergency system.

It was noted that both the occurrence of prodromal symptoms and the circumstances surrounding the acute attack were greatly similar in the comparison groups, and did not substantially contribute to differentiating the sudden death cases.

A high autopsy rate was achieved altogether 80.3 per cent of sudden coronary death cases, and the autopsy finding of recent myocardial infarction was surprisingly high. In a high percentage of the non-autopsied cases, the defined cause of death was supported by a previous symptomatic ischaemic heart disease. The cause of death remained uncertain in a few cases only.

In the general discussion the possibilities of preventing sudden coronary death and those of intervention with the aim of decreasing the number of sudden fatalities are discussed on the basis of the findings made in this study. Identification of the persons at the greatest risk of dying suddenly appeared to be difficult. It is also far from established what should be done for these persons if they could be identified.

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Haemodynamic Effects of Physical Training After Myocardial Infarction

By Arne Bjørnulf

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originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD. In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

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UNIVERSITY HOSPITAL UPPSALA SWEDEN

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The study was made on 63 male patients treated at the University Hospital, Uppsala, for acute myocardial infarction, with an onset during the period 15/3 1969–15/3 1972.

Patients with the following were excluded: previous cardiac infarction; diastolic blood pressure at rest of more than 110 mm Hg; a relative heart volume of more than 600 ml; manifest diabetes mellitus, other complicating diseases, e.g. pulmonary disease or arthritis of the hips or back. All patients equal to or more than 70 years of age were also excluded.

The 63 patients selected were admitted to hospital 3 months after the onset of the infarction for examination including right heart catheterization. They were then classified randomly to trained (34 patients) or reference group (29 patients).

Starting 3 months after the onset of the infarction, the patients of the trained group underwent a 3-month period of individual physical training (3 one-hour sessions per week under the leadership of a physiotherapist). The patients of the reference group carried out only their daily physical activity during this period (the reference period). Six months after the onset of the infarction the patients of both groups were readmitted to hospital for second examination including heart catheterization.

During the approximately 1000 training sessions no arrhythmias or other serious complications occurred. The emergency equipment that was always available (including defibrillator) was never needed.

The haemodynamic investigation showed that during work the pressure in the pulmonary circulation, including the PCV pressure, was pathologically increased. No change after the training period was noted. In both the trained and reference group lower heart rate at rest was recorded after the training or reference period. During work a decrease was noted in both groups, but this was more marked in the trained group. The stroke volume during submaximal work increased in the trained group, especially in the younger patients without angina pectoris. The cardiac output at rest and during work remained unchanged in both the trained and reference group. Neither was there any alteration of the arteriovenous oxygen difference. The left ventricular minute work was decreased after the training due to reduction of the arterial blood pressure during exercise. The reference group showed no significant reductions of left ventricular minute work or blood pressure. Finally the peripheral and central circulatory effects of the training, and their possible underlying mechanisms, were discussed.

Physical training in ischaemic heart disease is a form of treatment that has become increasingly common. During the last decade several authors have reported an increased physical work capacity, decreased severity of angina pectoris and an increased feeling of general well-being after a period of physical training (1, 13, 14, 16, 26, 35, 36, 45, 54, 55, 62, 68, 70). An important element of the beneficial effect of physical training — which is usually carried out in a group under the close supervision of a doctor — would seem to be psychological, resulting partly from a reduction of the anxiety and apprehension associated with physical effort. The beneficial effect probably also has an element with an objective basis, however. Different explanations for objective improvement of the physical work capacity are conceivable, e.g. increased myocardial performance (increased stroke volume in relation to the haemodynamic prerequisites), decreased work for the right or left ventricle per circulatory achievement (mainly through reduced pulmonary or peripheral resistance and possibly also through a change in the neurohumoral control of the myocardium), a reduced tendency to disturbing arrhythmias and, finally, a change of the metabolic control during muscular work.

Studies on the effect of physical training on the central and peripheral circulation in patients with coronary heart disease have been sparse and have only comprised a few patients, usually with no control group (16, 26, 27, 70). Mostly a decrease in the work of the heart in relation to the external work performed has been observed after training. Some studies (16, 17) have been concentrated mainly on effects on the peripheral circulation, while in others (25, 26, 27) more attention has been paid to changes in the central circulation.

The general aim of the present study was to compare the circulatory function after a period of physical training in part of a series of consecutive patients with myocardial infarction, with that in a reference group from the same series. These patients have usually an abnormal relationship between intracardiac pressure and flow. Firstly an attempt was made to find out if at least partial normalization of this relationship could be obtained. Secondly a study was made of the influence of age on the degree of measurable haemodynamic changes, and thirdly the question of whether the training mainly affected the central or the peripheral circulation was investigated.

ABSTRACT

The study was made on 63 male patients treated at the University Hospital, Uppsala, for acute myocardial infarction, with an onset during the period 15/3 1969–15/3 1972.

Patients with the following were excluded: previous cardiac infarction, a diastolic blood pressure at rest of more than 110 mm Hg, a relative heart volume of more than 600 ml, manifest diabetes mellitus, other complicating diseases, e.g. pulmonary disease or arthritis of the hips or back. All patients equal to or more than 70 years of age were also excluded.

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During the approximately 1000 training sessions no arrhythmias or other serious complications occurred. The emergency equipment that was always available (including defibrillator) was never needed.

The haemodynamic investigation showed that during work the pressure in the pulmonary circulation, including the PCV pressure, was pathologically increased. No change after the training period was noted. In both the trained and reference group lower heart rate at rest was recorded after the training or reference period. During work a decrease was noted in both groups, but this was more marked in the trained group. The stroke volume during submaximal work increased in the trained group, especially in the younger patients without angina pectoris. The cardiac output at rest and during work remained unchanged in both the trained and reference group. Neither was there any alteration of the arteriovenous oxygen difference. The left ventricular minute work was decreased after the training due to a reduction of the arterial blood pressure during exercise. The reference group showed no significant reductions of left ventricular minute work or blood pressure. Finally the peripheral and central circulatory effects of the training, and their possible underlying mechanisms, were discussed.

From both groups there was a considerable drop out amounting to a total of 21 out of 63 patients (13 in the trained group and 8 in the reference group). One patient (case 5) was excluded from the trained group because of lack of interest. Three patients (cases 6, 17 and 20) were excluded from the trained group and 4 (cases 6, 12, 16 and 26) from the reference group because they did not wish to undergo a second catheterization. None of the patients refused the first catheterization. In the trained group one reinforcement occurred (case 9), and another patient from that group (case 18) collapsed at home and died within the course of a few minutes, probably of infarction. One patient in the reference group (case 11) was excluded during the reference period (for definition see p. 9) because of increasing hypertension, which required active treatment with beta receptor blocking agents. One patient (case 24) was excluded from the trained group owing to an onset of ulcerative proctitis, which needed medical treatment and rest. Three patients (cases 11, 12 and 16), who were over 60 years old, were excluded from the trained group on account of deterioration with increasing severity of angina pectoris; case 16 also began to have breathlessness at rest. Two patients (cases 12 and 13) also developed pain in the hip joints and back. One patient (case 15) sustained a rupture of a thigh muscle at the first training session, but this only necessitated a two-week break in the training.

Three patients in both groups were excluded for technical reasons. During the Fick analysis in one patient in each group leakage was noted on collection of expired air. Case 3 in the trained group was excluded because at the second catheterization he discontinued his work before the work period was completed.

In one patient in the trained group (case 14) and 3 patients in the reference group (cases 5, 9 and 18) the catheter could not be advanced to the pulmonary artery and these patients were therefore also excluded from the calculations of the results, except case 5 for whom the cardiac output was calculated with the tip of the catheter lying in the right trunk at both catheterizations. The risk of error in the Fick analysis is somewhat greater with this catheter position (66). In 3 patients in the trained group (cases 2, 8 and 13) and in one patient in the reference group (case 11) the attempt to puncture the peripheral artery failed. In these patients the cardiac output was calculated on the assumption that the oxygen saturation both at rest and during work was 97.

Table 1. Age, height and weight in the trained and reference group after exclusions. There was a significant difference in height between the younger and older patients in the reference group ($P < 0.001$).

		Age (yrs)	Height (cm)	Weight (kg)
TRAINED GROUP				
Total	Mean	52.5	175	76.3
	SEM	1.75	1.49	1.69
	Range	35-68	164-195	64.0-91.5
	N	21	21	21
<55 yrs	Mean	48.4	176	76.8
	SEM	1.54	1.28	2.25
	Range	35-55	164-195	65.1-91.5
	N	14	14	14
>55 yrs	Mean	60.9	173	75.2
	SEM	1.67	2.49	2.49
	Range	56-68	165-183	64.0-80.5
	N	7	7	7

REFERENCE GROUP

Total	Mean	55.5	174	76.7
	SEM	2.02	1.14	2.13
	Range	36-69	165-184	60.6-98.0
	N	21	21	21
<55 yrs	Mean	47.9	178	77.7
	SEM	2.13	1.49	3.49
	Range	36-55	168-184	65.8-98.0
	N	10	10	10
>55 yrs	Mean	62.5	171	75.8
	SEM	1.34	1.05	2.68
	Range	56-69	165-175	60.6-90.0
	N	11	11	11

None of the patients were being treated with digitalis or beta receptor blocking agents during the training or reference period. After the exclusions there were 8 smokers in the trained group and 10 in the reference group.

In Table 1 the age, height and weight of the trained and reference groups are presented after the above exclusions. The two main groups were each divided into 2 subgroups: those 55 years of age or younger and those older than 55 years. The final trained group was on the average 3 years younger than the reference group but this difference was not significant. As can be seen in the table there were no significant differences between the main and subgroups with respect to height and weight, except between the reference subgroups. The number of patients in the main groups was 21.

The work tests were performed according to the method of Sjöstrand (64) and Wahlund (69) with an electrically braked ergometer bicycle (41), and with continuous ECG recording.

Training model

The patients of the reference group were given no instructions about increased physical activity or training; they were advised to take a daily walk, which should be increased gradually. This was in accordance with the routine followed in this hospital before 1969. The possibility that a reference patient might be told about the training by a patient in the trained group on meeting in hospital waiting rooms, for example, could hardly be prevented or excluded, but on no occasion did a reference patient ask to take part in the training programme.

The physical training was carried out 3 times a week at the hospital. Each training session was led by a qualified physiotherapist and lasted about 1 hour. Each patient was trained individually as the number of patients was too small to form a group.

Figure 3 shows the schematic arrangement of a training session. It started with 10 min rest, and this was followed by 10 min of calisthenics under the leadership of the physiotherapist, and then 3 ten-minute sessions of cycling on an ergometer bicycle with varying work loads. Between each cycling session the patient rested, sitting on the bicycle, for 3 min. The training ended with 10 min rest on a couch.

The patient was then allowed to wash his head and arms, but showering was not advised. It was considered desirable that the work load should reach but not exceed the pain threshold for the patients with angina pectoris. The highest heart rate in the training sessions towards the end of the training period was, on the average, $148 \pm (\text{SEM}) 2$ beats/minute for the younger patients (≤ 55 years) and 133 ± 4 for the older patients (> 55 years). The difference between these two groups was statistically significant ($P < 0.001$).

Of the 16 patients without angina pectoris in the trained group only 2 had chest pain during the training sessions: viz. case 26 (68 years old), who had pain in the chest on one occasion, and case 5 (45 years old), whose chest pain was associated with mild arrhythmia (3—4 isolated ventricular extrasystoles). The tendency to arrhythmia disappeared when a malfunctioning on-demand pacemaker had been removed. This patient then discontinued the training because of lack of interest.

Of the 15 patients in the trained group with angina pectoris the pain threshold was not reached in 2 cases: in case 13 there was a tendency to arrhythmia and despite a typical history of angina in case 34 and a heart rate of about 150 beats/minute during the training, this patient did not have chest pain during the training sessions either. This was possibly due to the fact that before each training session he had to walk up a slight hill to the hospital from the railway station. During this walk he almost always had a typical attack of angina pectoris. At the subsequent

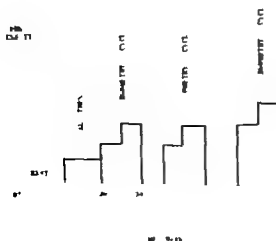


Fig. 3 Training design.

training session indoors after having been "warmed up" he therefore had no pain (second wind phenomenon). Because of severe angina pectoris the training was regarded as inadequate in case 27 in whom the highest heart rate during the training sessions did not exceed 130 beats/minute.

The training period comprised about 30 sessions. At each session the physiotherapist filled in a record card where she noted the carotid or radial pulse and the indirect brachial arterial pressure. The heart rate and blood pressure were recorded before and after the session and at the end of each 10 minute cycling period. Any unfavourable reaction, such as arrhythmia or congestion and decreasing blood pressure was also noted. A physician was always available in an adjacent room, where also emergency equipment including an ECG apparatus and a defibrillator were kept in readiness.

The patients were not connected to an oscilloscope during the training sessions.

Comments

In the approximately 1000 training sessions no serious complications such as reinfarction, cardiac arrest or tachyarrhythmias occurred. Thus the defibrillator never needed to be used, nor any of the other emergency equipment. The 2 cases of reinfarction did not occur during training, but in both cases about 24 hours after the last training session during this session no complications were noted.

The patients showed very positive attitude towards the physical training project as a form of treatment. Only one patient (case 4) lacked interest and did not attend the training sessions regularly.

In 2 patients (cases 6 and 27) the training was considered to be inadequate from a somatic point of view as the heart rate was kept too low during the training sessions for safety reasons.

Procedure for right heart catheterization

The day before the catheterization the patient visited the heart laboratory where he was told in detail about the procedure of the examination on the following day. After fasting overnight he was given 0.1 amobarbital, according to the routine 1 hour before the examination in the morning.

In 76 examinations a 70 mm long teflon catheter (I fart 115 mm, Stille-Werper, Stockholm) was introduced percutaneously into the radial artery. In

70 examinations the brachial artery was used instead, in which case a short polyethylene catheter (PE 160) was introduced by the Seldinger technique (63). Right heart catheterization was then performed in the usual way: the catheter was introduced through a small incision in a superficial medial cubital vein and was advanced to the pulmonary artery.

After the insertion of the 2 catheters and measurement of the pulmonary capillary venous (PCV) pressure the patient rested for 10 min. The cardiac output (CO) at rest was then determined by the direct Fick principle. Expired air was collected in a Douglas bag for 10 min (2.), after flushing of the bag and the tube system with expired air for a few min. The heart rate was noted every other min from the ECG and the pressure in the systemic artery (SA) and the pulmonary artery (PA) was noted. Between the 5th and the 6th min blood samples were taken simultaneously from SA and PA for measurement of the arterio-venous oxygen difference ($AVDO_2$).

When the tip of the catheter had been placed in the PCV position, CO was determined during exercise, the patient cycling in the supine position for 6 min with the feet strapped to an electrically braked bicycle ergometer (Elema-Schöander, Stockholm). The mean work load was 320 kpm/min (52 W) (range 100–500 kpm/min) for the trained group and 296 kpm/min (49 W) (range 150–500 kpm/min) for the reference group. This difference was not significant. The work load was set at approximately half of the highest attained load at the work tests of the previous days. ECG and intra-arterial pressures were recorded continuously on direct writing recorder (Mingograf 81).

The expired air was collected during the last 3 min of the examination. During the first 3 min the tip of the catheter was in the PCV position, between the 3rd and 4th min it was withdrawn to PA and between the 4th and 5th min blood samples were taken simultaneously from PA and SA for determination of $AVDO_2$.

Seven patients had chest pain during exercise during the catheterization procedure, 3 of them at both examinations.

At the second heart catheterization each patient had the same work load as at the first catheterization.

Comments

No severe complications, such as ventricular fibrillation occurred during the heart catheterizations, and

neither was there any attack of ventricular tachycardia. On the other hand, 2 cases in the reference group (cases 2 and 18) showed transient atrial fibrillation which regressed after i.v. administration of 0.4 mg Cedilanid.[®] Both of these attacks of atrial fibrillation occurred when the tip of the catheter was in the right atrium. An impression was obtained that these post-infarction patients did not have a greater tendency to extrasystoles during the manipulations of the catheter than patients with other cardiac diseases.

As mentioned above in 76 of the catheterizations the radial artery was used and in 20 the brachial artery. In 14 cases different arteries were used at the two examinations. In 5 of these cases (including 2 from the trained group) a higher blood pressure (>5 mmHg) was noted in the radial artery in 4 cases (including 2 from the trained group) the blood pressure was higher in the brachial artery and in 5 cases (including one from the trained group) the blood pressures in the two arteries were the same. It was therefore considered of no importance for measurement of the blood pressure which of these arteries was used.

For measurement of CO the direct Fick method was used. The volume of expired air was measured with a gasometer (Nordgas Stockholm) and the used air was analysed according to the method of Ldane with some modifications (21). Duplicate determinations were performed in the gas analyses and the error of a single determination in this laboratory was ± 0.2 vol% for oxygen and ± 0.1 vol% for carbon dioxide. The values for oxygen uptake (V_{O_2}) were compared with the normal values obtained from Harris & Benedict (32).

The oxygen saturation was determined spectrophotometrically on haemolysed whole blood (43-44) the error of a single determination was 0.2% with an oxygen saturation of about 75%. The haemoglobin concentration was measured spectrophotometrically after conversion to cyanmethaemoglobin. The error of a single determination was ± 0.02 g%. The oxygen capacity was determined from the haemoglobin concentration under the assumption of an oxygen-binding capacity of 1.39 ml O₂/g Hb (19-67). The oxygen content was obtained from the values for oxygen saturation and oxygen capacity with the addition of physically dissolved oxygen. AVO₂ is the difference in oxygen content in the blood between SA and PA.

Fick's equation gives the cardiac output through

the formula: $\text{oxygen uptake ml/min} = \text{cardiac output l/min} \times \text{arteriovenous oxygen difference ml/l}$

Wranne (73) found in this laboratory an error of 6% in a determination by the direct Fick method, obtained in duplicate determinations of the cardiac output in 10 cases.

The cardiac index (CI) (l/min·m²) was calculated by dividing cardiac output by body surface area as obtained from Du Bois & Du Bois (18).

Pressures were measured by pressure transducers of the capacitance type (EMT 34 or 35 Elema-Schönander Stockholm). These were connected to amplifier units and an electromanometer (EMT 31 Elema-Schönander Stockholm). The pressure curves and ECG were recorded on an eight-channel Mingograf 81 and the mean pressures were obtained by electrical integration. The paper speed was 25 mm per sec except when the end-diastolic pressure of the right ventricle was measured when it was 50 mm/sec.

The reference level for zero pressure in the supine position was placed 5 cm dorsal to the sternal insertion for the fourth rib. Calibrations were performed with a water manometer before each examination.

The pulmonary vascular resistance (PVR) was calculated from the ratio

$$\frac{PA_m - PCV_m}{CO}$$

and expressed in mm Hg/l/min. CO = cardiac output, PA_m = pulmonary arterial mean pressure, PCV_m = mean pulmonary capillary venous pressure.

The left ventricular minute work (LVMW) (kpm/min) was calculated from the formula

$$1 \text{ LVMW} = \dot{Q} \times \text{MABP} \times 0.0144 \text{ kpm/min}$$

where \dot{Q} = cardiac output, MABP = mean arterial blood pressure and 0.0144 = the product of the specific density of blood, 1.056 g/ml, and that of mercury 13.6 g/ml (10), divided by 1000 to convert mm into m.

The left ventricular stroke work (LVSW) was calculated from the formula

$$\text{LVSW} = \text{SV} \times \text{MABP} \times 0.0144 \text{ g}\cdot\text{m}$$

where SV = stroke volume (ml)

Statistical methods

(in collaboration with Bernhard Hulfeldt)

A General concepts and definitions.

The following definitions of statistical concepts have been used

i) Mean $M = \frac{1}{N} \sum x_i$ N = number of observations.

ii) Variance $VAR = \frac{1}{N-1} \sum (x_i - M)^2$

iii) Standard deviation $SD = \sqrt{VAR}$

iv) Standard error of the mean $SEM = \frac{SD}{\sqrt{N}}$

v) Slope $\frac{y_2 - y_1}{x_2 - x_1}$

B. Statistical analysis of the difference between values at rest and during work.

i) The change in individual slope

Bivariate observations were made concerning various physiological variables, e.g. stroke volume and oxygen uptake. This was done both before and after the training or reference period and in each instance both at rest and during work. Individual slopes were calculated, assuming one of the variables to be the dependent one (see Fig. 8). The mean and standard deviation of the differences between individual final and initial slopes were then calculated both in the trained and in the reference group. Each of the means was tested for significance by a *t*-test for paired differences described in any statistical textbook (37). This gave an indication of whether any change had occurred in the relationship between the two studied variables from the first examination to the second. With the two variables mentioned above as an example, the question was whether for a given oxygen uptake (amount of work) the stroke volume increased on the average between the two examinations.

ii) Comparison between trained and reference group

To find out whether the trained group had gained more physiological capacity during the inter-

vening period, the means of the slope differences in the two groups were compared in a test of significance. The method used was Student's *t*-test for differences in independent means.

C. Statistical analysis of the differences between values after and before the training or reference period. The effect of training was analysed by studying the difference in mean change for different variables in two groups. This was done in a multiple regression analysis with an indicator variable for classification group, values before training, age, place of residence (urban/rural), and occurrence of angina pectoris were used as other independent variables. This procedure makes the comparison relatively efficient as influences on the differences in mean changes from those variables mentioned above will be eliminated. The coefficient for the indicator variable was tested for significance by means of Student's *t*-test.

*D. In other comparisons between the trained and reference group Student's *t*-test was used, but for comparisons of changes within each group and for each patient only a *t*-test for paired differences.*

E. Significance levels.

The term significant is used in accordance with the following convention. All tests are tested at the 5 % level of significance. If an observed deviation from the null hypothesis is of such a magnitude that the probability P of obtaining a deviation at least as great as the observed one is greater than 0.05 (here the null hypothesis is assumed to hold), then the observed deviation is consequently said to be non-significant and is designated as. If P is less than 0.05 the deviation is said to be significant.

The degree of significance has been given, using the following notation.

If $0.001 < P < 0.05$ the significance is denoted by $P < 0.05$

If $0.001 < P < 0.01$ the significance is denoted by $P < 0.01$

If $P < 0.001$ the significance is denoted by $P < 0.001$

RESULTS

In the following values obtained before the training or reference period are called initial values and those obtained after those periods are called final values.

Circulation at rest

In Table II some data are given at rest. The mean ventilation was normal in both groups and there were no significant differences either before or after the training or reference period. Case 21 in the trained group had considerably increased values on both occasions, 17.8 and 11.9 l/min. The reason for this is not clear but his oxygen uptake was also somewhat high. However there were no signs of hyperthyroidism or pronounced anxiety. Case 31 in the trained group and cases 3 and 24 in the reference group had values exceeding 10 l/min both before and after the training and reference period. These high values in relation to the others were considered to be due to apprehension.

The initial oxygen uptake was $245 \pm (\text{SEM}) 9$ ml/min or 6% above the predicted basal oxygen uptake in the trained group. The corresponding value for the reference group was 240 ± 8 ml/min or 4% above the predicted basal value. The final values showed no significant changes and there were no significant differences between the age groups.

The mean arterial oxygen saturation was normal in all groups and there were no significant differences between initial and final values. In the trained group one patient (case 1) with no manifest pulmonary disease or signs of right to left shunt had a value of 91.8 at the first examination. His value after training was 94.6.

The arteriovenous oxygen difference showed no significant changes after the training or reference period. No significant differences were found between either the main groups or the age groups. Four patients in the trained group and two patients in the reference group had values exceeding 60 ml. Three of these patients were older than 60 years or had angina pectoris.

The mean cardiac output showed no significant changes after the training or reference period. In both main groups the younger patients had larger values than the older ones; the difference was significant in the trained group for both the initial values ($P < 0.02$) and the final ones ($P < 0.05$). This difference was due to the higher \dot{V}_{O_2} and lower AVDO_2 in the younger patients. Two older patients in the trained group (cases 6 and 26) had values of 3.0 l/min or lower.

The cardiac index reflected the cardiac output, but there were no significant differences in this respect.

From Table III it can be seen that the mean stroke volume was not significantly changed in the trained or reference group. In both groups there was a tendency to lower values in the older patients. The final value for the older reference patients was, however, significantly higher than the initial one.

The mean heart rate lay between 50 and 90 beats/min in all patients. Both in the trained and reference group there was a significant decrease from initial to final values. The changes were also significant in the age groups except for the older patients in the trained group, for whom the final value was the same as the initial one.

The mean pulmonary vascular resistance was normal both before and after the training or reference period. There were no significant changes or differences.

The left ventricular minute work decreased in both main groups after the training or reference period, but the changes were not significant. Neither were there any significant changes in left ventricular stroke work except in the older patients of the reference group, where the initial value was 97 ± 8 and the final value 118 ± 5 g m ($P < 0.05$).

Pressures

Table II gives the pressures in the right heart and the PCV position. All recorded pressures lay within normal limits. There were no significant changes except for the older patients in the trained group, in whom the PCV pressure decreased from 8 ± 0.8 to 5 ± 0.3 mm Hg.

Table III. Heart-beat numeric data at rest before and after job, and training or reference period (B)

Stroke volume (ml)		Heart rate (beats/min)		Pulmonary vas. resist. (mm Hg/min)		Left ventricular minuteme work (kgm/min)		Left ventricular stroke work (g·cm)	
Before	After	Before	After	Before	After	Before	After	Before	After
TRAINED GROUP									
T 14									
Mean	77	67 P<0.001		64	1.0	1.2	7.5	6.9	110
SEM	3.30	1.82		1.73	0.22	0.33	0.46	0.39	5.13
Range	48-110	52-85		49-77	0.2-2.4	0.5-2.9	3.9-12.7	4.7-11.1	81-177
N	22	23		21	18		18		18
<55 yrs									
Mean	80	68 P<0.001		63	1.0	1.0	8.1	7.2	117
SEM	4.28	2.41		2.41	0.16	0.12	0.37	0.49	7.38
Range	58-110	52-85		49-77	0.2-2.1	0.5-2.1	6.0-12.7	5.3-11.1	89-177
N	15	16		14	12		12		12
>55 yrs									
Mean	70	65		65	1.2	1.6	6.3	6.3	97
SEM	4.57	2.14		1.91	0.22	0.26	0.54	0.62	10.3
Range	48-84	54-71		59-72	0.7-2.4	0.8-2.9	3.9-7.8	4.7-8.4	81-130
N	7	7		7	6		6		6
REFERENCE GROUP									
Total									
Mean	76	70 P<0.001		65	1.1	1.2	7.6	7.3	107
SEM	3.51	1.87		1.69	0.13	0.16	0.60	0.31	7.74
Range	49-111	53-88		46-78	0.2-2.0	0.0-2.7	4.0-14.7	5.3-10.7	60-210
N	21	21		20	19		19		19
<55 yrs									
Mean	75	71 P<0.05		67	1.0	1.2	8.5	7.3	118
SEM	5.51	2.81		2.33	0.18	0.19	0.98	0.53	13.1
Range	63-111	60-88		57-76	0.3-1.9	0.5-2.3	5.3-14.7	5.3-10.7	82-201
N	10	10		10	9		9		9
>55 yrs									
Mean	68 P<0.05	77		63	1.3	1.1	6.8	7.3	97 P<0.01
SEM	4.21	3.33		2.39	0.17	0.27	0.67	0.37	8.07
Range	49-92	51-93		46-78	0.2-2.0	0.0-2.7	4.0-11.8	5.5-9.0	60-152
N	11	11		10	10		10		10

Table IV. Haemodynamic pressure data (mm Hg) at rest before and after the physical training or reference period (4). There was significant difference ($P < 0.05$) between the final PCW values of the two older groups.

		RA Mean		RV Systolic		RV End-diastolic		PCV Mean	
		Before	After	Before	After	Before	After	Before	After
TRAINED GROUP									
Total	Mean	3	3	23	24	3	3	8	7
	SEM	0.38	0.38	0.78	0.74	0.45	0.46	0.57	0.44
	Range	-2.7	0.7	18-36	16-30	-2.7	-2.7	5-16	4-11
	N	23		23		23		22	
≤55 yrs									
Mean	3	3	23	24	3	3	8	8	
SEM	0.45	0.48	0.58	0.77	0.57	0.61	0.78	0.47	
Range	-3.6	0.6	19-26	16-30	-2.7	-2.7	5-16	4-11	
N	16		16		16		15		
>55 yrs									
Mean	4	4	24	23	4	3	8 $P<0.01$	5	
SEM	0.48	0.59	2.25	1.74	0.40	0.64	0.71	0.25	
Range	3-7	2-7	18-36	16-29	3-6	1-6	6-28	4-28	
N	7		7		7		7		
REFERENCE GROUP									
Total	Mean	2	2	22	24	3	3	7	7
	SEM	0.33	0.40	1.11	0.89	0.50	0.25	0.62	0.41
	Range	-1.4	0.6	16-36	17-34	-2.6	-2.6	1-11	4-10
	N	21		20		19		20	
≤55 yrs									
Mean	2	3	22	23	3	3	7	6	
SEM	0.47	0.51	1.37	0.99	0.64	0.37	0.53	0.54	
Range	-1.4	0.6	16-28	17-27	0-5	2-5	6-9	4-10	
N	10		10		9		10		
>55 yrs									
Mean	2	2	23	23	3	3	7	7	
SEM	0.48	0.38	1.80	1.43	0.77	0.34	1.16	0.62	
Range	-1.4	0.4	18-36	20-34	-2.6	2-5	1-11	4-10	
N	11		10		10		10		

($P < 0.01$). There was also a significant difference between the final values in the two older age groups ($P < 0.05$).

Table 5 presents the pressures in the pulmonary and systemic arteries. All pulmonary arterial pressures lay within normal limits and there were no significant changes or differences. The same applies to the

systemic artery, except for the mean pressure in the older patients of the reference group, which rose from 97 ± 2 to 107 ± 3 mm Hg ($P < 0.05$). The final systolic and mean pressures of the trained group were significantly lower than the corresponding pressures of the reference group due to differences between the older age group.

Circulation during exercise

The ventilation (Table VI) decreased significantly in both main groups after the training or reference

period. In the reference group there was also a significant decrease in the younger age group.

The oxygen uptake during exercise represented an approximately four-fold increase from the resting

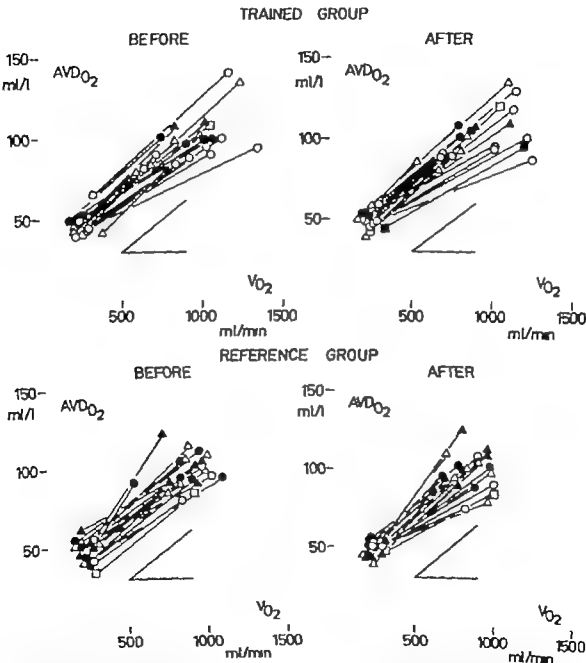


Fig. 4 Arteriovenous oxygen difference ($AVDO_2$) in relation to oxygen uptake (V_{O_2}) at rest and during work on the supine position for 21 trained and 21 reference patients before and after the training or reference period.

Open symbols represent patients ≤ 55 years of age, and filled symbols patients > 55 years of age. Triangles represent

patients with angina pectoris and squares represent patients with questionable angina pectoris. Circles represent patients without angina pectoris.

The mean slope of the line rest-work is indicated in each of the four sub figures.

Table V. (Mean/max/min) pressure data (mm Hg) at rest before and after physical training or reference period (B). After the training or reference period there was significant difference ($P < 0.01$) in the systolic SA pressure between the two main groups. Also the diastolic pressure in the older age groups was significantly different ($P < 0.01$). There was also significant difference ($P < 0.05$) in mean pressure in the two main groups after the training or reference period.

										Pulmonary artery				Systemic artery			
										Systolic		Diastolic		Mean		Systolic	
										Before	After	Before	After	Before	After	Before	After
TRAINING GROUP																	
Total	N	22	21	21	13	13	8	8	8	136	133	88	88	97	96	73	73
Mean		145	145	145	135	135	135	135	135	136	133	88	88	97	96	73	73
SEM		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Range		110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	80-135	80-135	60-110	60-110
N		22	21	21	13	13	8	8	8	136	133	88	88	97	96	73	73
≤ 55 yrs	Mean	145	145	145	135	135	135	135	135	136	133	88	88	97	96	73	73
	SEM	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Range	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	80-135	80-135	60-110	60-110
	N	22	21	21	13	13	8	8	8	136	133	88	88	97	96	73	73
> 55 yrs	Mean	145	145	145	135	135	135	135	135	136	133	88	88	97	96	73	73
	SEM	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Range	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	80-135	80-135	60-110	60-110
	N	22	21	21	13	13	8	8	8	136	133	88	88	97	96	73	73
REFERENCE GROUP																	
Total	N	20	20	20	12	12	7	7	7	138	148	77	77	101	105	76	78
Mean		145	145	145	135	135	135	135	135	138	148	77	77	101	105	76	78
SEM		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Range		110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	80-135	80-135	60-110	65-100
N		20	20	20	12	12	7	7	7	138	148	77	77	101	105	76	78
≤ 55 yrs	Mean	145	145	145	135	135	135	135	135	138	148	77	77	101	105	76	78
	SEM	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Range	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	80-135	80-135	60-110	65-100
	N	20	20	20	12	12	7	7	7	138	148	77	77	101	105	76	78
> 55 yrs	Mean	145	145	145	135	135	135	135	135	138	148	77	77	101	105	76	78
	SEM	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Range	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	110-170	80-135	80-135	60-110	65-100
	N	20	20	20	12	12	7	7	7	138	148	77	77	101	105	76	78

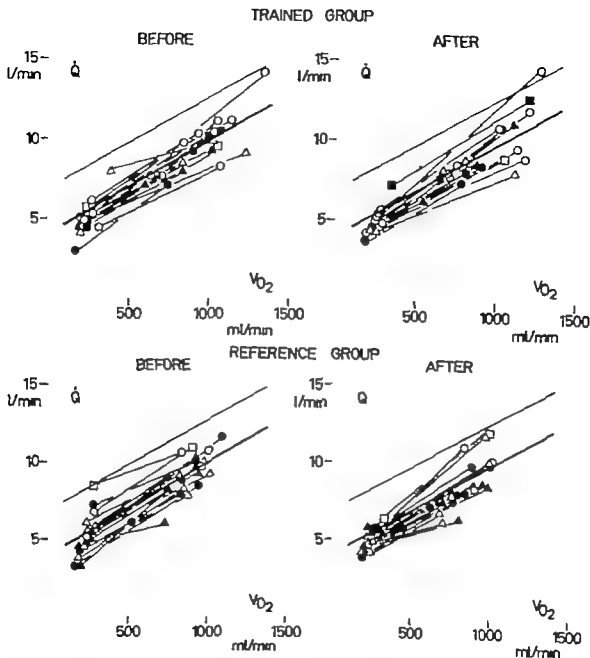


Fig. 5 Cardiac output (\dot{Q}) in relation to oxygen uptake (\dot{V}_{O_2}) at rest and during work in the supine position for 11 trained and 21 reference patients before and after the training or reference period. Symbols as in Figure 4.

blue. There were no significant changes after the training or reference period or differences between groups. The younger patients of both groups had a higher oxygen uptake than the older ones.

The arterial oxygen saturation was normal and remained essentially unchanged after the training or

The heavy line represents the regression line during exercise for healthy men aged 61–83 (Gronlund *et al.* (29)) and the thin line the corresponding regression line for healthy men aged 23–41 (Holmgren *et al.* (40)).

reference period in the two groups. Also in the subgroups small and insignificant changes were noted. The mean arteriovenous oxygen difference showed no significant changes after the training or reference period. Neither were there any significant differences between the main or age groups. Figure 4

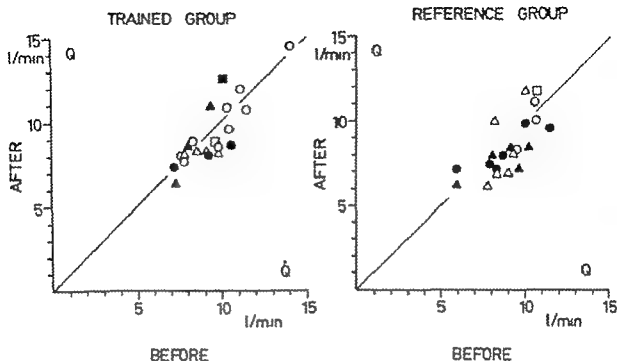


Fig. 6. Cardiac output (Q) during work before and after the training air reference period in 21 trained and 21 reference patients. Symbols as in Figure 4.

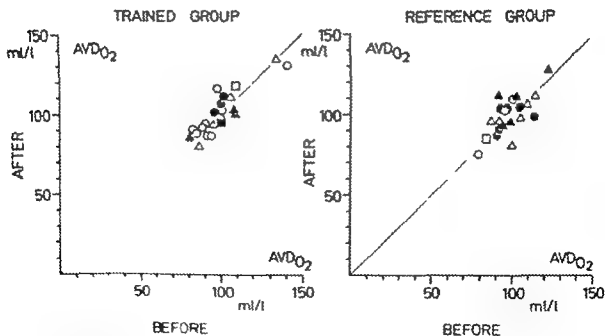
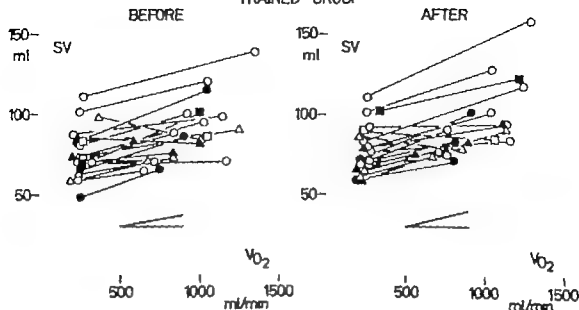


Fig. 7. Arteriovenous oxygen difference ($AVDO_2$) during work before and after the training air reference period in 21 trained and 21 reference patients. Symbols as in Figure 4.

TRAINED GROUP



REFERENCE GROUP

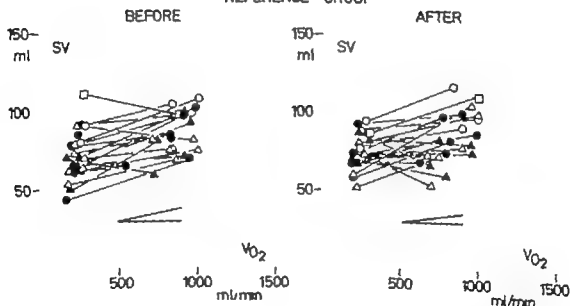


Fig. 8 Stroke volume (SV) related to oxygen uptake ($\dot{V} = \dot{V}O_2$) at rest and during exercise in 21 trained and 11 reference patients before and after the training or reference period. Symbols as in Figure 4.

After training there was significant ($P < 0.02$) increase of the mean slope of the rest-work line.

illustrates how $\Delta \dot{V}O_2$ increased in all patients with increasing oxygen uptake. There were no significant changes between the mean slopes (rest-work) either before or after the training or reference period.

Neither were there any significant differences between the trained and reference group.

The cardiac output showed no significant changes after the training or reference period, compared with

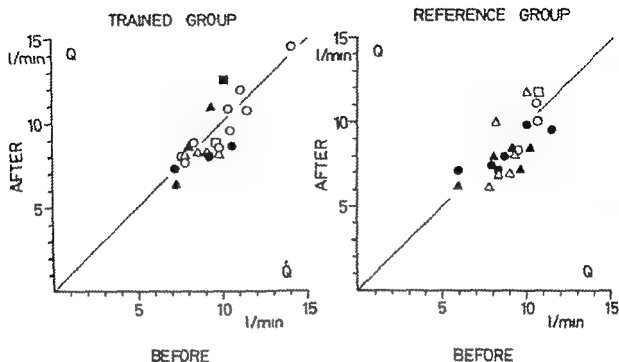


Fig 6 Cardiac output (Q) during work before and after the training or reference period in 21 trained and 21 reference patients. Symbol as in Figure 4

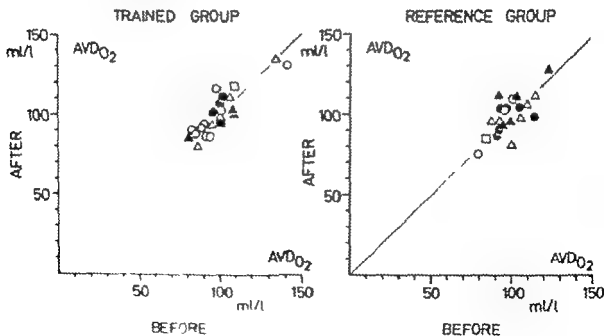


Fig 7 Arteriovenous oxygen difference (AVDO₂) during work before and after the training or reference period in 21 trained and 21 reference patients. Symbol as in Figure 4

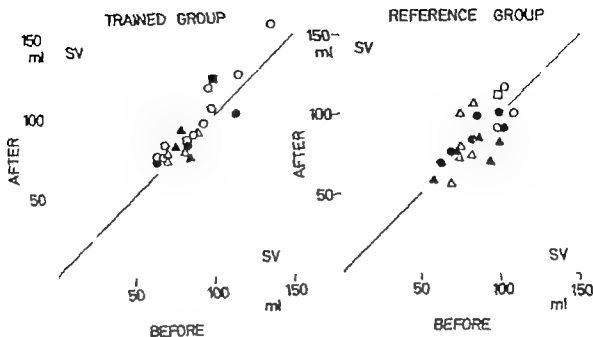


Fig. 10 Stroke volume (SV) during work before and after the training or reference period in 21 trained and 1 reference patients. Symbols as in Figure 4.

before. The younger group had larger cardiac outputs than the older ones in both groups, but there were no significant differences. In Figure 5 it is seen how the cardiac output increased with increasing oxygen uptake in all patients. The patients are compared with healthy men aged 23–41 years (40) and 61–83 years (79).

In Figure 6 it is seen that the cardiac output of the younger patients remained unchanged after training, also in the older patients and those with angina pectoris there was no clear tendency towards a lower cardiac output at the end of the training period. The two older patients with an increased cardiac output after training (cases 18 and 30) were 58 and 59 years old, respectively. The patient with angina pectoris had it very mildly and had a maximal heart rate of about 140 beats/min at the training sessions.

The cardiac index was normal in both groups and no significant changes were observed after the training or reference period.

Figure 7 presents the arteriovenous oxygen difference before and after the training or reference period. It can be seen that there were no changes of AVD_{O_2} after the training period.

In the trained group there was a significant increase of the stroke volume (Table VII), which was

more pronounced in the younger patients. The largest increase was noted in the 9 younger patients without angina pectoris, from 93 to 103 ml ($P < 0.01$). The reference group showed no significant changes of SV. The younger patients of both groups had larger stroke volumes than the older ones, but these differences were not significant.

Figure 8 illustrates the relation between SV and V_{O_2} under conditions of rest and exercise. The mean slope of the rest work line was significantly increased after the training period ($P < 0.05$). In the reference group there was no significant change.

Figure 9 presents the relation between SV and HR at rest and during exercise. Here also the slope was significantly increased ($P < 0.01$) after the training period. In the reference group there was no significant change. A significant difference was found between the two main groups after the training or reference period ($P < 0.05$).

In Figure 10 it can be seen that the stroke volume increment of the trained group was due mainly to the younger patients, who initially showed a larger value than the others. The filled square symbol indicates a 42-year-old man (case 18) with questionable angina pectoris. In the reference group the picture is less clear. On multiple regression analysis, with the

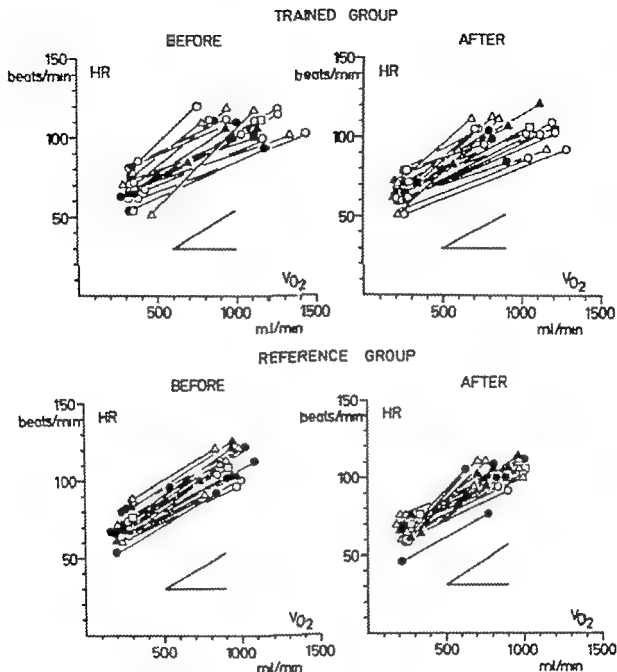


Fig. 11 Heart rate (HR) related to oxygen uptake (V_{O_2}) at rest and during work in the supine position before and after the training or reference period in 21 trained and 21 reference patients. Symbols as in Figure 4.

After training there was a significant decrease ($P < 0.01$) of the mean slope of the rest-work line in the trained group. There was also a significant difference ($P = 0.001$) in slope between the two groups after the training or reference period.

Table VII. Hammond mean flow data at work before and after the physical training or reference period (R).

Stroke volume			Heart rate		Pulmonary vec.		Left ventricular		Left ventricular	
(ml)			(beats/min)		revol. (mm Hg/min)		stroke work (kg/min)		stroke work (g m)	
	Before	After	Before	After	Before	After	Before	After	Before	After
TRAINED GROUP										
Total	88	94	107	110	1.3	1.1	17.5	16.3	155	155
Mean	88	94	107	110	1.3	1.1	17.5	16.3	155	155
SEM	4.11	4.37	2.15	2.07	0.18	0.13	0.66	0.67	9.34	8.53
Range	65-138	72-159	85-120	83-120	0.0-3.1	0.2-2.5	11.4-24.7	10.7-22.8	103-238	104-218
N	21	22	22	22	18	18	17	17	17	17
<55 yrs	89	95	110	110	1.2	2.0	18.3	16.2	167	161
Mean	89	95	110	110	1.2	2.0	18.3	16.2	167	161
SEM	5.47	6.39	2.35	2.11	0.20	0.10	1.09	0.90	11.5	10.4
Range	65-138	74-159	88-120	86-110	0.4-3.1	0.4-1.6	13.1-24.7	12.5-21.3	111-238	120-218
N	14	15	15	15	12	12	11	11	11	11
>55 yrs	87	90	103	99	1.4	1.1	16.1	14.6	155	144
Mean	87	90	103	99	1.4	1.1	16.1	14.6	155	144
SEM	6.26	6.82	4.23	4.96	0.37	0.34	1.61	1.86	16.9	14.8
Range	65-115	72-124	85-117	83-120	0.0-2.5	0.3-2.5	11.4-20.6	10.7-22.8	103-224	104-190
N	7	7	7	7	6	6	6	6	6	6
REFERENCE GROUP										
Total	85	85	107	107	1.0	1.1	16.5	16.2	156	159
Mean	85	85	107	107	1.0	1.1	16.5	16.2	156	159
SEM	3.19	3.71	2.23	1.98	0.14	0.16	0.81	0.67	8.00	7.01
Range	63-109	56-117	92-122	76-114	0.0-2.3	0.4-2.7	11.1-23.2	11.8-21.4	111-247	105-227
N	21	21	21	21	18	18	18	18	18	18
<55 yrs	87	90	109	103	1.3	1.1	17.2	16.4	159	160
Mean	87	90	109	103	1.3	1.1	17.2	16.4	159	160
SEM	4.53	4.28	3.00	2.43	0.17	0.15	1.10	1.05	13.4	11.6
Range	75-109	56-117	96-122	91-112	0.5-2.5	0.4-1.5	13.9-25.2	11.8-31.4	124-247	105-227
N	18	18	10	10	9	9	9	9	9	9
>55 yrs	83	80	104	102	0.7	1.1	15.7	15.9	152	159
Mean	83	80	104	102	0.7	1.1	15.7	15.9	152	159
SEM	4.43	3.91	3.24	3.18	0.18	0.23	1.20	0.90	9.45	8.75
Range	63-103	68-101	92-122	76-114	0.0-1.7	0.5-2.7	11.1-20.0	12.7-21.0	111-200	122-194
N	11	11	11	11	9	9	9	9	9	9

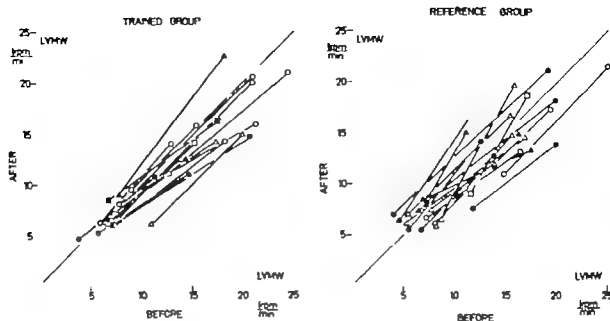


Fig. 12. Left ventricular minute work (LVMW) at rest and during work before and after the training or reference period in 17 trained and 18 reference patients. Symbols as in Figure 4

change of the stroke volume during exercise after the reference or training period on the y axis and the initial value during exercise on the x axis, using age, residence and presence or absence of angina pectoris as independent variables, no significant change of the stroke volume was found after the training or reference period.

The mean heart rate (Table VII) decreased slightly but significantly in both main groups. This was due to the younger patients because in the older patients the heart rate was essentially the same before and after the training or reference period. Figure 11 shows the relation between HR and \dot{V}_{O_2} under conditions of rest and exercise. In the trained group a significantly smaller slope (rest work) was noted after the training period ($P < 0.01$). In the reference group there was no significant change. There was a significant difference ($P < 0.001$) between the main groups after the training or reference period.

The pulmonary vascular resistance was normal before and after the training or reference period, and no significant differences were found between the groups (Table VII).

Table VIII also presents data for the left ventricular minute work. After training there was a significant decrease ($P < 0.01$), while in the reference group the final value was essentially the same as the initial value.

The decrease of LVMW was achieved mainly because of the reduction of the mean arterial blood pressure after training. In Figure 12 it is seen that all trained patients except three had a lower value after the training. The patient with the largest increase (case 30) was 59 years old and his increase was due to an increment of the cardiac output from 9.3 to 11.3 l/min. In the reference group both increases and decreases were noted, but of the 7 patients with an increase all belonged to the older age group or had angina pectoris, except one patient (case 22).

Multiple regression analysis, with the change of LVMW during exercise after the training or reference period on the y axis and the initial value during exercise on the x axis, using age and the presence or absence of angina pectoris as independent variables, showed no significant change of LVMW after the training or reference period.

The left ventricular stroke work showed no significant changes or differences between either the main groups or the age groups. Lower values were observed in the older patients of both groups.

Pressures

Table VIII presents data for the pulmonary arterial and pulmonary capillary pressures. The PA

Table VIII. *Hemodynamic pressure data (mm Hg) at work before and after the physical training or reference period. The final values for the diastolic pressure in the pulmonary artery differed significantly between the two younger groups ($P < 0.05$)*

		Pulmonary artery (PA)						PCV	
		Systolic		Mean		Diastolic		Mean	
		Before	After	Before	After	Before	After	Before	After
TRAINED GROUP									
Total	Mean	46	46	33	33	22	22	26	24
	SEM	2.49	3.05	2.25	2.26	1.43	1.54	2.25	2.02
	Range	27-70	23-80	15-58	14-58	10-32	8-38	5-45	7-45
	N	22		22		22		19	
<55 yrs	Mean	47	46	33	34	21	22	25	24
	SEM	3.37	4.01	3.03	2.94	1.74	1.78	3.20	2.78
	Range	27-70	23-80	15-58	14-58	10-30	8-35	5-45	7-45
	N	15		15		15		13	
>55 yrs	Mean	52	47	38	33	25	22	28	24
	SEM	3.87	4.66	2.69	3.62	2.37	3.11	2.44	2.48
	Range	45-67	35-70	27-47	24-50	16-32	16-38	20-37	16-32
	N	7		7		7		6	
REFERENCE GROUP									
Total	Mean	43	44	31	31	20	20	21	21
	SEM	3.06	2.51	2.67	2.07	1.94	1.61	2.71	2.12
	Range	18-66	30-70	12-50	18-48	7-35	10-34	8-46	9-45
	N	19		19		19		19	
<55 yrs	Mean	40	39	28	26	19	16	17	17
	SEM	3.48	2.47	3.20	1.87	2.61	1.26	2.16	1.85
	Range	23-55	31-55	14-45	18-36	9-35	10-22	8-28	9-28
	N	9		9		9		10	
>55 yrs	Mean	46	48	34	35	21	23	25	25
	SEM	4.88	3.83	4.12	2.97	2.94	3.56	4.93	3.51
	Range	18-66	30-70	12-60	22-48	7-34	10-34	10-46	15-45
	N	10		10		10		9	

pressures were abnormally increased in comparison with healthy old men 61—81 years (29). A tendency to higher pressures in the older than in the younger patients of the two groups was noted. There were no significant changes before or after the training or reference period.

The mean PCV pressure was also abnormally increased in comparison with that in healthy old men (29). A tendency to higher pressures in the older age groups. No significant changes in the PCV pressure were noted after the training or reference period.

The *systemic arterial pressures* are presented in Table IX. On comparison with healthy old men (29) the pressures lay at the upper normal limit at a corresponding work load. In the reference group there were no significant changes but in the trained group there was a significant decrease of both the *systolic mean and diastolic pressures*. The pressures of case 25 in the reference group were excluded from the calculations because they probably were not correct (at the first examination his systolic pressure was 115 mm Hg and at the second examination it was 250 mm Hg).

Table IX. Pressures (mm Hg) in the systemic artery at work before and after the physical training or reference period. After the training or reference period there were significant differences between the main groups both in the systolic ($P<0.05$) mean ($P<0.01$) and diastolic ($P<0.05$) pressures. The final values for the older patients in each main group also differed significantly ($P<0.05$), except those for systolic pressure.

		Systolic		Mean		Diastolic	
		Before	After	Before	After	Before	After
TRAINED GROUP							
Total	Mean	183 $P<0.05$	172	124 $P<0.01$	115	88 $P<0.05$	82
	SEM	6.15	5.36	3.83	3.67	2.46	3.16
	Range	150-240	145-220	105-165	90-140	70-110	60-110
	N	18		18		18	
<55 yrs	Mean	183	173	125	115	90	88
	SEM	8.13	6.20	5.38	3.96	3.39	4.10
	Range	150-240	150-210	105-165	90-135	70-110	60-110
	N	12		12		12	
>55 yrs	Mean	181	171	123 $P<0.05$	108	86	78
	SEM	9.75	11.8	4.62	7.74	3.01	6.02
	Range	150-210	145-220	110-135	90-140	75-90	70-100
	N	6		6		6	
REFERENCE GROUP							
Total	Mean	183	188	124	128	91	92
	SEM	6.62	7.12	4.00	4.35	2.88	3.04
	Range	145-255	150-270	100-165	110-180	75-115	80-120
	N	19		18		19	
<55 yrs	Mean	173	174	126	121	93	89
	SEM	10.7	7.53	5.42	3.05	3.63	3.41
	Range	145-255	150-225	110-165	110-135	80-115	80-105
	N	9		9		9	
>55 yrs	Mean	192	201	123	136	89	96
	SEM	7.46	10.5	6.18	7.48	4.46	4.85
	Range	160-250	160-270	100-160	110-180	75-110	80-120
	N	10		9		10	

In Figure 13 it is seen that in all trained patients except three there was a reduction of the mean arterial pressure after the training period. In at least one of these (case 13 with initial slope 110 and final slope 130 mm Hg) the training intensity was not very high because of angina pectoris and a tendency to premature ectopic beats during exercise. In the reference patient both increases and decreases are recorded. The three cases with the greatest increase (cases 1, 17 and 79) were all more than 60 years old and had angina pectoris.

Multiple regression analysis, with the difference between the mean arterial blood pressure during exercise after and before the training or reference period on the y-axis and the blood pressure during exercise before these periods on the x-axis, and with age and the presence or absence of angina pectoris as independent variables, showed a significantly lower blood pressure in the trained group as compared with the reference group ($P<0.01$).

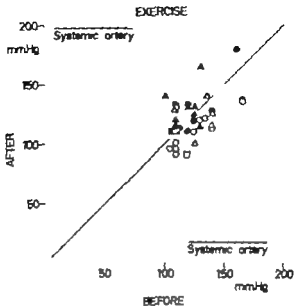


Fig. 13 Systemic arterial (SA) pressure during work before and after the training or reference period in 18 trained and 18 reference patients.

Open symbols represent patients of the trained group, and filled symbols patients of the reference group. Triangles represent patients with angina pectoris and squares patients with questionable angina pectoris. Circles represent patients without angina pectoris.

Table IX. Pressures (mm Hg) in the systemic artery at work before and after the physical training or reference period. After the training or reference period there were significant differences between the main groups both in the systolic ($P<0.05$), mean ($P<0.01$), and diastolic ($P<0.05$) pressures. The final values for the older patients in each main group also differed significantly ($P<0.05$), except those for systolic pressure.

		Systolic		Mean		Diastolic	
		Before	After	Before	After	Before	After
TRAINED GROUP							
Total	Mean	183 $P<0.05$	172	124 $P<0.01$	113	88 $P<0.05$	83
	SEM	6.15	5.36	3.83	3.67	2.46	3.16
	Range	150-240	145-220	105-165	90-140	70-110	60-110
	N	18		11		11	
<55 yrs	Mean	183	173	125	115	90	88
	SEM	8.13	6.20	5.38	3.96	3.39	4.10
	Range	150-240	150-210	105-165	90-135	70-110	60-110
	N	12		12		12	
>55 yrs	Mean	182	171	123 $P<0.05$	108	86	81
	SEM	9.75	11.8	4.62	7.74	3.01	6.02
	Range	150-210	145-220	110-135	90-140	75-90	70-100
	N	6		6		6	
REFERENCE GROUP							
Total	Mean	183	188	124	128	91	92
	SEM	6.62	7.12	4.00	4.35	2.88	3.04
	Range	145-255	150-270	100-165	110-180	75-115	80-120
	N	19		18		19	
<55 yrs	Mean	173	174	126	121	93	89
	SEM	10.7	7.53	5.42	3.05	3.63	3.41
	Range	145-255	150-225	110-165	110-135	80-115	80-105
	N	9		9		9	
>55 yrs	Mean	192	201	123	136	89	96
	SEM	7.46	10.5	6.18	7.48	4.46	4.85
	Range	160-250	160-270	100-160	110-180	75-110	80-120
	N	10		9		10	

In Figure 13 it is seen that in all trained patients except three there was a reduction of the mean arterial pressure after the training period. In at least one of these (case 13 with initial value 110 and final value 130 mm Hg) the training intensity was not very high because of angina pectoris and tendency to extracardiac beats during exercise. In the reference patients both increases and decreases were recorded. The three cases with the greatest increase (cases 1, 13 and 19) were all more than 60 years old and two had angina pectoris.

Multiple regression analysis, with the difference between the mean arterial blood pressure during exercise after and before the training or reference period on the y axis and the blood pressure during exercise before these periods on the x axis, and with age and the presence or absence of angina pectoris as independent variables, showed a significantly lower blood pressure in the trained group as compared with the reference group ($P<0.01$).

support the view that the increase in work capacity and reduction of cardiac work after physical training in patients with coronary artery disease are due to effects on the peripheral circulation.

Frick (25-26), on the other hand, observed no significant decrease of the cardiac output or increase of the arteriovenous oxygen difference during submaximal work after training of a group of six patients with earlier myocardial infarction, three of whom had angina pectoris (mean age 48 years). The increase of the stroke volume that he observed has been ascribed to improved contractility and muscular hypertrophy (24).

A reason for the varying results of the physical training can be differences in the *examination procedure* as some authors have examined the patients in the sitting posture (16-20) and others with the patient supine (26-27). The supine posture gives a more hyperkinetic state with optimal conditions for filling of the heart chambers, greater venous return, a larger cardiac output, a higher blood pressure, a lower heart rate and a greater stroke volume. With this posture a stroke volume increase after physical training is probably more easily obtained (39).

It seems that both *age* and the question of whether or not *angina pectoris* is present are of importance for the effect of the training. In the present series there was an increase of the stroke volume only in the younger age group of the trained patients. This

stroke volume increase during submaximal exercise after training was still more pronounced in the younger patients without angina pectoris. Whether this fact only reflects the lower training intensity in the older patients and those with angina pectoris or whether there are other differences also cannot be stated on the basis of the present results. In cases with previous infarction and old age or angina pectoris more severe coronary heart disease can be presumed.

Perhaps it may be assumed that the old infarction patient, especially if he has had a large infarct, has little possibility of increasing his stroke volume during exercise after training because of the damaged myocardium. In that case there will possibly be a compensatory increase of the peripheral circulatory effects after a period of physical training. The divergent results in the above mentioned studies (17-27) on a small series of patients could then be explained by inhomogeneity of the series, i.e. larger infarcts in the series with mainly peripheral effects (17-21), and younger patients with no angina pectoris in the series with mainly central circulatory effects (27). All patients in the series with mainly peripheral effects also had angina of effort. In the study with mainly central effects only half (three of six) had angina pectoris. The patients were also slightly younger in this latter study.

CONCLUSIONS

During the approximately 1000 training sessions no arrhythmias or other serious complications occurred. The risk involved with this form of treatment can thus be said to be very small. The interest in and the rate of adherence to the treatment was very high among the patients, and only one of 34 discontinued the training for lack of motivation. Of the six patients whose training was terminated because of reinfarction, other disease, increasing severity of angina pectoris or breathlessness, five were 60 years old or older. This indicates that the older patient has smaller reserves than the younger and therefore more easily suffers over-exertion. The haemodynamic effect of the training was also less pronounced in the older group of patients (more than 55 years). No significant reduction of the work of the heart was observed in this group nor any increase of the stroke volume during exercise. On the other

hand the mean arterial blood pressure during exercise was significantly lower after the training. In the younger group of patients (less than or equal to 55 years) there was a reduction of the heart rate, an increase of the stroke volume, a decrease of the mean arterial blood pressure and LVMW during exercise after the training period. The abnormally high pressures in the pulmonary circulation during work were unchanged after the training and reference period.

The objective improvement that takes place in the infarct patient after a period of physical training can be caused both by effects on the central and on the peripheral circulation. It is possible that the central effect is not so easily achieved in older patients, patients with angina pectoris or those with large infarcts. These patients therefore conceivably have a compensatorily larger effect on the peripheral circulation.

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Table XIX. Left ventricular minute work (LVW) (lpm/min) at rest and during exercise before and after the training or reference period.

TRAINED GROUP					REFERENCE GROUP				
Rest		Exercise			Rest		Exercise		
Case	Before	After	Before	After	Case	Before	After	Before	After
1	12.7	11.1	24.7	21.2	1	6.3	7.3	11.1	15.0
3	7.6	7.4	—	—	2	5.6	5.5	12.5	14.0
4	7.2	5.3	14.0	12.5	3	7.6	9.0	—	—
7	8.0	9.5	15.5	15.9	4	7.2	6.8	16.4	13.2
10	5.8	5.2	14.7	11.0	5	7.1	7.9	16.2	14.7
15	6.5	6.4	21.0	20.7	7	14.7	10.7	25.2	21.4
18	8.8	8.4	17.5	16.3	10	8.5	6.3	15.6	14.8
19	7.8	6.7	21.3	20.0	13	4.4	6.5	11.5	16.5
21	6.0	6.2	13.1	14.0	14	7.5	7.5	16.7	14.5
22	6.7	6.3	15.2	14.1	17	6.8	8.5	17.3	13.1
25	7.0	6.1	20.6	14.7	19	5.3	7.3	15.4	16.5
28	3.9	4.7	11.4	10.7	20	8.2	5.3	15.7	19.7
29	6.6	5.6	14.4	12.4	21	11.8	7.7	20.0	17.8
30	7.8	8.0	18.1	22.8	22	11.5	9.0	17.0	18.7
31	11.2	5.4	20.0	15.3	23	8.2	6.5	19.3	17.3
32	7.8	8.2	21.2	15.9	24	5.3	6.6	13.9	11.8
33	7.4	5.9	18.2	14.1	27	7.4	8.3	19.9	18.2
34	7.6	7.5	17.3	14.4	28	6.7	5.5	13.8	12.7
Mean	7.5	6.9	17.5	15.6	29	4.0	7.1	19.4	21.0
SEM	0.46	0.39	0.86	0.87	Mean	7.6	7.3	16.5	16.2
Range	3.9-12.7	4.7-11.1	11.4-24.7	10.7-22.8	SEM	0.60	0.31	0.81	0.67
N	18		17		Range	4.0-14.7	5.3-10.7	11.1-25.2	11.8-21.4
					N	19		18	

Table XX. Left ventricular stroke work (LVSW) (g-m) at rest and during exercise before and after the training or reference period.

TRAINED GROUP					REFERENCE GROUP				
Rest		Exercise			Rest		Exercise		
Case	Before	After	Before	After	Case	Before	After	Before	After
1	177	154	238	206	1	101	111	111	140
3	104	108	—	—	2	105	120	136	185
4	101	88	133	120	3	91	126	—	—
7	105	123	139	169	4	114	111	171	144
10	81	78	135	114	5	104	120	158	138
15	107	110	184	204	7	201	178	247	227
18	98	116	175	161	10	97	84	130	135
19	135	135	209	218	13	67	108	124	171
21	89	99	111	132	14	91	98	137	135
22	98	103	136	133	17	94	125	169	224
25	130	102	224	176	19	76	117	140	166
26	82	75	103	104	20	126	78	132	177
29	93	81	137	118	21	152	127	200	182
30	121	118	156	190	22	152	118	157	176
31	135	115	167	140	23	127	114	196	171
32	124	144	192	159	24	82	104	124	105
33	157	118	185	165	27	112	133	178	165
34	91	97	145	129	28	84	82	133	132
Mean	110	109	163	155	29	60	125	159	194
SEM	6.25	5.13	9.34	8.53	Mean	107	115	156	159
Range	81-177	78-154	103-238	104-218	SEM	7.74	5.05	8.00	7.01
N	18		17		Range	60-201	78-178	111-247	105-227
					N	19		18	

Table XXL Pressures in the right atrium (RA) and right ventricle (RV) (mm Hg) at rest before and after the training or reference period.

TRAINED GROUP					REFERENCE GROUP								
RA			RV		RA			RV					
Case	Mean Before	After	Systolic Before	After	End-diastolic Before	After	Case	Mean Before	After	Systolic Before	After	End-diastolic Before	After
1	3	4	22	16	5	0	1	2	1	36	29	6	3
2	3	5	25	22	4	6	2	2	1	18	25	1	3
3	3	5	25	25	4	3	3	4	2	25	23	6	4
4	3	1	23	23	1	2	4	3	3	23	22	4	2
7	-2	0	22	22	-2	-2	5	2	3	-	-	-	-
8	4	6	25	25	7	7	7	1	3	19	23	0	3
10	4	2	20	23	4	1	8	3	3	16	26	1	3
13	2	2	25	23	3	4	10	3	6	21	24	0	5
15	2	2	20	28	3	6	13	-1	4	15	20	-2	2
18	7	7	24	16	6	3	14	2	4	25	27	2	5
19	3	3	19	25	3	6	17	2	2	20	25	3	3
21	3	3	26	30	0	4	19	4	3	16	19	-	-
22	2	2	24	22	0	0	20	4	2	28	17	5	3
23	5	5	22	27	3	5	21	3	0	21	20	3	1
25	5	5	25	20	3	3	22	-1	0	20	24	3	3
26	3	3	18	19	3	2	23	3	1	28	24	5	2
27	4	4	27	29	4	3	24	2	2	20	20	3	4
29	4	4	21	26	4	6	25	4	1	26	34	4	5
30	4	4	36	26	5	5	27	4	2	23	24	5	3
31	6	6	26	24	6	3	28	0	2	20	20	2	2
32	1	1	24	24	3	3	29	3	4	24	28	2	3
33	4	4	20	22	5	4		-	-				-
34	1	1	20	24	1	3	Mean	2	2	22	24	3	3
Mean	3	3	23	24	3	3	SEM	0.33	0.40	1.11	0.89	0.50	0.25
SEM	0.38	0.38	0.78	0.74	0.45	0.46	Range	1-4	0-6	16-36	17-34	-2-6	2-6
Range	2-7	0-7	18-36	16-30	-2-7	-2-7	N	21		20		19	
N	23		23		23								

Table XXII. Pressures in the pulmonary artery (PA) (mm Hg) at rest before and after the training or reference period.

TRAINED GROUP						REFERENCE GROUP											
Systolic			Mean			Diastolic			Systolic			Mean			Diastolic		
Case	Before	After	Before	After	Before	After	Case	Before	After	Before	After	Before	After	Before	After		
1	19	15	12	10	8	6	1	29	19	15	10	9	6				
2	21	16	14	11	10	8	2	17	19	9	10	5	6				
3	22	23	14	12	7	6	3	17	15	10	10	7	6				
4	26	20	17	13	11	8	4	27	25	15	13	8	8				
7	15	16	8	9	2	6	7	17	19	9	12	5	8				
8	16	19	11	15	7	11	8	19	20	11	12	7	8				
10	20	17	12	10	6	5	10	22	25	14	18	8	11				
13	23	21	14	13	7	5	13	13	25	6	13	3	9				
15	17	18	10	11	6	5	14	21	27	15	18	9	7				
18	28	18	19	11	14	7	17	24	28	13	15	8	8				
19	16	24	10	16	7	8	19	15	18	10	10	6	8				
21	25	27	18	18	12	12	20	20	17	14	9	8	5				
22	24	23	14	12	7	6	21	22	20	13	12	7	7				
23	17	16	12	12	8	8	22	12	14	—	—	5	6				
25	23	20	13	11	6	6	23	18	20	14	12	8	7				
26	16	18	10	10	6	6	24	14	13	9	9	5	7				
27	20	22	12	11	7	5	25	27	19	11	12	7	5				
29	20	26	12	14	6	10	27	24	18	16	12	10	8				
30	25	28	16	15	8	10	28	16	17	9	11	5	9				
31	24	20	14	13	9	9	29	27	30	18	19	12	11				
32	26	26	13	20	6	14											
33	22	22	14	14	8	8	Mean	20	20	12	13	7	7				
34	29	25	13	14	7	9	SEM	1.13	1.09	0.71	0.70	0.47	0.38				
Mean	22	21	13	13	8	8	Range	12-29	13-30	6-18	9-19	5-10	5-11				
SEM	0.85	0.81	0.55	0.58	0.51	0.51	N	20		19		20					
Range	15-29	15-28	8-19	9-20	2-14	5-14											
N	23		23		23												

Table XXIII. Pressures in the pulmonary artery (PA) (mm Hg) during exercise before and after the training or reference period.

TRAINED GROUP						REFERENCE GROUP											
Systolic			Mean			Diastolic			Systolic			Mean			Diastolic		
Case	Before	After	Before	After	Before	After	Case	Before	After	Before	After	Before	After	Before	After		
1	33	38	24	26	18	19	1	66	70	46	48	32	34				
2	47	40	37	31	25	20	2	33	45	18	32	11	21				
4	54	40	43	30	27	18	3	58	50	44	39	28	20				
7	77	23	15	14	10	8	4	49	44	30	31	22	21				
8	33	29	20	22	13	15	7	23	32	14	20	9	10				
10	48	42	37	27	23	18	8	36	43	24	28	14	16				
13	43	39	26	25	14	17	10	38	57	28	26	20	16				
15	48	45	35	15	22	25	13	18	38	12	26	7	18				
18	45	35	33	24	23	16	14	44	40	32	28	21	18				
19	48	52	28	36	13	23	17	43	41	31	30	22	1				
21	70	73	52	53	30	35	19	38	33	24	22	18	18				
22	68	80	58	58	30	32	20	55	36	45	24	35	16				
23	46	47	38	37	29	27	21	46	40	39	25	17	10				
24	50	40	35	27	16	18	22	26	31	17	18	11	12				
26	37	38	27	26	18	16	23	45	55	37	36	25	22				
27	46	46	46	36	20	22	25	46	60	35	44	20	3				
29	55	56	40	40	30	28	27	54	50	43	40	28	24				
30	67	70	47	50	32	38	28	30	30	20	22	12	13				
31	38	36	24	28	18	18	29	64	60	50	46	34	32				
32	60	60	40	42	23	28											
33	42	36	28	28	16	18	Mean	43	44	31	31	20	20				
34	44	54	32	38	22	20	SEM	3.06	2.51	2.67	2.07	1.84	1.61				
							Range	18-66	30-70	12-50	18-48	7-35	10-34				
Mean	48	46	35	33	22	22	N	19		19		19					
SEM	2.49	3.05	2.25	2.26	1.43	1.54											
Range	27-70	23-80	15-58	14-58	10-32	8-38											
N	22		22		22												

Table XXIV Pulmonary capillary mean venous PCV pressures (mm Hg) at rest and during exercise before and after training or reference period.

TRAINED GROUP					REFERENCE GROUP				
Rest			Exercise		Rest			Exercise	
Case	Before	After	Before	After	Case	Before	After	Before	After
1	8	7	15	18	1	8	4	46	45
2	8	9	23	18	2	4	7	15	25
3	5	8	—	—	3	9	10	33	33
4	16	9	37	27	4	6	8	19	20
7	2	4	5	7	7	7	8	8	17
8	9	9	14	16	8	8	7	12	17
10	8	5	37	25	10	8	10	19	16
13	7	7	—	—	13	1	6	11	17
13	6	7	29	28	14	5	6	11	13
18	8	5	20	16	17	4	5	15	18
19	8	10	23	23	19	6	5	15	16
21	—	—	40	40	20	9	4	28	10
22	10	8	45	45	21	5	6	17	13
23	7	6	26	27	22	4	7	10	9
25	7	6	25	22	23	9	5	24	28
26	6	5	—	—	24	8	6	24	24
27	9	5	28	20	25	11	10	—	—
29	8	4	25	32	27	10	8	36	36
30	12	6	32	30	28	4	8	10	18
31	11	10	17	22	29	12	7	46	24
32	7	9	—	—	Mean	7	7	21	21
33	8	11	24	18	SEM	0.62	0.41	2.71	2.12
34	9	6	20	28	Range	1-11	4-10	8-46	9-45
Mean	8	7	26	24	N	20		19	
SEM	0.57	0.44	2.25	2.02					
Range	2-16	4-11	5-45	7-45					
N	22		19						

Table XXV. Pressures in the systemic artery (SA) at rest before and after the training or reference period (mm Hg)

TRAINED GROUP				REFERENCE GROUP			
Systolic		Mean		Systolic		Mean	
Case	Before After	Before After	Diastolic	Case	Before After	Before After	Diastolic
1	195 170	150 135	110 100	1	160 155	100 110	70 75
3	110 140	80 90	60 70	2	145 150	95 100	70 70
4	150 130	100 90	80 70	3	145 165	100 120	85 95
7	130 160	100 120	75 100	4	130 135	100 100	75 80
10	120 110	85 80	70 65	5	135 130	100 100	80 70
13	135 150	100 110	75 80	7	210 185	150 130	100 100
15	120 120	85 90	65 70	10	130 150	100 110	80 90
18	145 125	100 80	75 60	13	140 170	95 110	70 80
19	130 125	85 80	60 60	14	130 125	100 100	80 80
21	120 115	90 90	70 70	17	140 140	100 100	80 75
22	110 110	80 80	60 65	19	120 130	100 100	75 80
25	155 140	110 100	80 80	20	120 130	95 90	70 70
26	130 135	90 90	70 70	21	100 140	115 95	80 70
29	120 125	90 95	75 75	22	130 130	95 95	75 75
30	140 145	100 105	70 80	23	135 120	110 90	85 70
31	145 130	95 95	70 60	24	120 130	90 95	70 70
32	150 150	100 110	70 80	25	140 185	95 130	70 90
33	130 110	90 80	70 60	27	150 165	90 105	60 65
34	140 140	105 95	80 70	28	135 150	90 100	70 75
Mean	136 133	97 96	73 73	29	135 165	90 110	70 70
SEM	4.51 3.92	3.55 3.40	13.2 11.8	Mean	138 148	101 105	76 78
Range	110- 110-	80- 80-	60- 60-	SEM	4.76 4.41	2.97 2.56	3.09 2.13
	195 170	150 135	110 100	Range	100- 120-	90- 90-	60- 65
N	19	19	19		210 185	150 130	100 100
				N	20	20	20

Table XXVI. Pressures in the systemic artery (SA) during exercise before and after the training or reference period (mm Hg)

TRAINED GROUP				REFERENCE GROUP			
Systolic		Mean		Systolic		Mean	
Case	Before After	Before After	Diastolic	Case	Before After	Before After	Diastolic
1	240 210	165 135	110 95	1	195 235	130 165	100 120
4	175 170	125 110	90 70	2	185 190	110 130	75 80
7	160 185	110 130	85 110	3	180 175	— —	110 100
10	160 145	110 95	80 70	4	160 170	120 110	90 80
13	150 180	110 130	75 90	5	160 175	110 120	80 100
15	190 175	130 120	90 85	7	235 225	165 135	115 105
18	195 160	105 95	85 60	10	160 180	120 130	95 100
19	185 170	105 95	70 60	13	200 200	100 140	80 100
21	160 150	110 110	85 85	14	170 150	125 120	100 80
22	150 160	110 110	90 85	17	180 180	125 125	95 90
25	210 180	135 120	90 80	19	160 160	130 115	90 85
26	150 150	110 100	75 70	20	145 180	110 115	80 80
29	175 170	125 100	90 90	21	200 200	140 125	90 85
30	200 220	135 140	95 100	22	160 150	110 110	80 80
31	220 200	140 125	95 90	23	170 180	125 120	100 90
32	200 160	140 115	100 80	24	175 170	125 130	90 100
33	170 130	110 90	80 70	27	190 220	120 130	75 80
34	200 180	140 115	105 85	28	180 160	110 110	75 80
Mean	183 172	124 113	88 82	29	250 270	160 180	110 120
SEM	6.15 5.36	3.83 3.67	2.46 3.16	Mean	183 188	124 128	91 92
Range	150- 145	105- 90-	70- 60-	SEM	6.62 7.12	4.00 4.35	2.88 3.04
	240 220	165 140	110 110	Range	145 150-	100- 110-	75 80-
N	18	18	18		253 270	165 180	115 120
				N	19	18	19

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Ischaemic Heart Disease in Women

**A study based on a randomized population sample of women
and women with myocardial infarction
in Göteborg, Sweden**

By Calle Bengtsson

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A study based on a randomized population sample of women
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By Calle Bengtsson

in collaboration with

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TOTAL GENERAL POPULATION

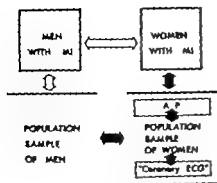


Fig 1 Possible comparisons (arrows) within the material discussed in the present report. Filled arrows denote comparisons made in the present investigation. AP = angina pectoris; coronary ECG = ECG changes suggestive of IHD.

population between men with MI and women with MI and between subjects with different manifestations of IHD and the general population. As shown by the filled arrows in Fig 1, the present study of IHD in women aims to compare women with and without IHD in the population sample, to compare women with MI registered in the MI register and women in the population sample, and to compare men and women in the respective population samples in order to find answers to two questions:

1. Are there differences between women with MI or other manifestations of IHD in the general population and 'healthy' women in the same general population?
2. Are there differences in the prevalence of risk factors between men and women in the general population which can explain the lower incidence of IHD in women?

MATERIAL AND METHODS

The data of the present monograph derive from a population study of women in Göteborg¹⁵ and from the Post Mortal Clinic in Göteborg^{20 21} Göteborg is the second largest city in Sweden. Its situation on the west coast and had about 445000 inhabitants in 1968. Further details concerning Göteborg are given elsewhere.¹²

The Population Study of Women in Göteborg 1968-1969

Performance The basic data of the present investigation were obtained from a population study of women performed in Göteborg, Sweden in 1968-1969¹⁵. A sample of women in the age strata 38-46, 50-54 and 60 (born in 1930-1922, 1918-1914 and 1908) totaling 1462 women was studied. Women born on dates which were even multiples of six (6, 12, 18, 24, 30) were called for the study. In the age group 54 only those who were born on the 6th or on the 12th of every month were called and in the age group 60 only those born on the 6th. The sample was obtained from the Regional Office Register. Those born at the beginning of the year were called first. The survey was performed for the most part during a 12-month period. In this way the influence of age differences within each age group was reduced as far as possible.

The participation rates of the various age groups are shown in Table I. The overall participation rate was 90.1%.

The examination of the population sample took place at Sahlgren Hospital in Göteborg. There a checklist was filled in during the whole examination period. The women came fasting in the morning and were interviewed and examined at different examination stations according to a special schedule. A study of cardiovascular disease was one of the main research projects of the investigation. Haematological, gynaecological and psychiatric studies were also performed as well as a project on peripheral diabetes. The same examiner performed the same examination during the whole study period. A more complete list of search projects is given elsewhere.¹⁵

Non participation The number of refusals and other non-participants is shown in Table I. Single women were to some extent over-represented among the refusals while there was no difference of importance between the participants and the refusals with regard to place of birth.⁵ Telephone interviews with most non-participants together with studies of records from inpatient and outpatient clinics gave further information about 93% of the refusals.¹¹ In this way information was obtained from altogether 99.2% of those who were still living in Göteborg at the time of the examination. It was found that there was a difference of importance between the participants and the refusals with regard to heart disease¹¹ or blood pressure.¹

Tabl I Participants and non participants in th Population Study of Women in Götting 1968-1969

Age (year)	Called for examination	Non participants			Refused	Participants	Participation rate (%)
		Dead	Moved	Inaccessible			
38	407	3	7	1	24	372	91.4
46	479	0	8	2	38	431	90.1
50	436	3	4	0	31	398	91.0
54	203	2	0	1	20	180	88.6
60	97	0	1	0	15	81	83.5
Total	1622	8	20	4	128	1462	90.1

Causes of death 1950-1969 Death certificates were obtained from the Central Bureau of Statistics Stockholm for all women who had died in 1950 or later and who because of the sampling method would have been invited to the examination if still alive. Forty-eight women had died during the period 1950-1967. Another 8 women died in 1968-1969 during the interval between the sampling and the intended examination date. The diagnoses of these 56 women are shown in Table II. The diagnosis was based on autopsy findings in at least 38 of the women (68%). Only a small number had died from heart disease. This agrees with observations from a previous similar study of cause of death in men.¹⁸⁷

Table II Causes of death of 56 women who died between 1950-1969 and who because of the sampling method would have been invited to take part in the population study if still alive

Cause of death	n	Cause of death	n
Heart disease	6	Liver disease	1
Aortic disease	1	Illness after childbirth	1
Cerebrovascular disease	2	Myoma of the uterus	1
Thromboembolic disease	2	Tuberculosis	1
Malignant hypertension	1	Systemic collagen disease	1
Kidney disease	5	Nervous system disease	4
Diabetes	2	Suicide	8
Cancer	15	Accident	4
Leukemia	1		

Ischaemic heart disease in the population sample

Myocardial infarction The diagnosis of myocardial infarction (MI) was considered established when it had been verified in hospital

Angina pectoris Symptoms of angina pectoris (AP) were recorded by interview according to the questionnaire proposed by Rose¹⁵⁵ AP was defined according to Rose¹⁵⁵ as chest pain or discomfort with the site including either the sternum or the left arm together with the left anterior chest. This pain is provoked by either hurrying or walking uphill (or by walking on the level for those who never attempt more). When it occurs on walking it should make the subject either stop or slowen pace until some nitroglycerin is taken. It should disappear in the majority of occasions within 10 minutes or less from the time that the subject stands still.

ECG changes suggestive of ischaemic heart disease (IHD). An ECG examination was performed after overnight fasting with the subject in the supine position on a couch. A trained nurse took the ECG after the subject had been resting for about 10 minutes. A direct writing recorder (Mingograf 42 B, Elma Schönande AB, Stockholm, Sweden) was used. Leads I, II, III, aVR, aVL, aVF, GR1, GR2, GR4, CR5 and CR7 were recorded. The ECGs were interpreted by an experienced technician (Mr. Dana Vrobova, Prague) using the Minnesota Code¹⁵⁶ with the 'Scandinavian modification' with out access to other information about the subjects. Minnesota Codes 1:1 2 4 1 5 1 2 (in the absence of 3 1) 6:1 7 1 were defined as coronary ECG.

The Post MI Clinic in Göteborg

Registration Subjects born in 1913 or later who live in Göteborg and who have had an acute MI have been registered at the Post MI Clinic since the 1st of January 1968⁵⁰. The registration has been carried out under the supervision of the Section for Preventive Cardiology at Sahlgren's Hospital, Göteborg. The intention has been to register all men and women with an acute attack of MI.

Subjects having a MI in Göteborg are taken to the emergency department of Sahlgren's Hospital, which is the only hospital for acute medical care of heart disease in Göteborg. An initial registration is made at the emergency department. All subjects with suspected MI are followed up by the registering personnel until the diagnosis of MI is proved or disproved. In addition, all medical wards are visited by the registering personnel twice a week in order to register subjects whose diagnosis of MI might have been missed at the emergency department.

Criteria of MI The criteria of acute MI have been: Central chest pain of more than 15 minutes duration with onset during 48 hours and/or frank pulmonary oedema without previously known

heart disease and/or shock without suspicion of acute hypovolemia or intoxication together with

a) appearance of a pathological Q wave and/or appearance or disappearance of a localized ST elevation followed by a T inversion in two or more of the leads

and/or

b) two SGOT values above the upper limit for normal variations of the laboratory¹⁷ with a maximum level about 24 hours after onset of symptoms in combination with low or SGPT values with a maximum level after about 36 hours

The criteria agree with those recommended by the Swedish Heart Society

The registration of the subjects with symptomatic MI has been supplemented by studying all death certificates issued since the 1st of January 1968

Further details about the registration of symptomatic MI, incidence of MI and incidence of death from IHD are given elsewhere⁶

Performance of the interviews and examinations. The subjects with MI were interviewed and examined in a standardized way by one of the physicians according to special forms. The methodology was generally the same as in the population studies of men^{18,2} and women¹⁵ in Göteborg. When there are differences in methodology they will be mentioned in the separate chapters. The women were examined at the time of the acute attack and at clinical visits 3, 12 and 24 months after the attack³¹. In addition the women were examined by the authors on an extra occasion in order to obtain complementary and supplementary data.

Women with myocardial infarction registered at the Post MI Clinic

Survivors on arrival in hospital. The present investigation concerns women in Göteborg who were born in 1913 or later and who had an acute attack of MI during the years 1968-1970. Those who left hospital as survivors were admitted to the standardized follow-up study with clinical visits 3, 12 and 24 months after the MI. One woman aged 23 with systemic lupus erythematosus had been excluded from the study as her MI was considered to be a complication of her pre-existing disease.²³

Death. The death certificates of all women in Göteborg who were born in 1913 or later and who had died during the years 1968-1970 were examined. Further data about these deaths were obtained from inpatient records and autopsy reports.

Women who died suddenly (death within the first few hours before reaching hospital) were considered to have died from MI when autopsy revealed a fresh MI or when acute severe chest pain had preceded death.¹⁷

Unexpected deaths were classified as coronary deaths when the women were not known to have had chest pain preceding death, and autopsy revealed severe coronary atherosclerosis but no fresh MI area.^{II}

Previous studies on men

A population study of 855 50 year old men in Göteborg was carried out in 1963^{III} and these men were re-examined in 1967 when they were 54 years old. The men were representative for the general male population in the same way as the women in the present study were representative for the general female population in Göteborg. Consequently comparisons may be made between men aged 50 and 54 and women in the present study aged 50 and 54 in order to look for differences between men and women in the general population of these age groups.

Method for comparing women with IHD and women in the general population

The parametric variables of the women in the population sample were divided into deciles when data from all the age groups studied were available. The women with IHD were then placed in the deciles of these variables according to the decile limit for their age in the population study. In this way a comparison could be made between the women with IHD and the women in the population sample. The whole population sample then constituted the reference group. Comparison could also be made with the general population as the population sample was representative for the general female population. The values of the women with IHD were expected to lie within the upper deciles if there was an association between IHD and high values of the variable studied.

The non-parametric variables of the women with IHD were usually compared with those of the women of the ages 50 and 54 in the population sample who had about the same mean age as the women with IHD. Table III shows mean age, standard deviation (SD) and range in the different groups of women with IHD and in the group of women from the population study aged 50 and 54 who thus usually constituted the reference group when studying the non-parametric variables. For special reasons other reference groups were chosen when studying gluco-tolerance and insulin response to intravenous glucose injection^{IV} and when studying stress factor and behavioural traits.^I The exceptions will be discussed separately in the chapters which concern these matters.

The comparisons were made between the women in the different IHD groups and the women in the reference group who were free from the IHD manifestation studied.

Tabl III Mean age S.D and range (years) in th groups of women with MI AP and coronary ECG and in the participants of th population tudy aged 50 and 54 who usually constituted the reference group

Cat gory	n	M an age	S.D	Rang
Women with MI				
Survivors on arrival in hospital	47	52.2	4.5	37-57
Deaths outside hospital	10	50.5	5.3	40-56
Women with AP	29	51.3	4.8	38-60
Women with coronary ECG"	23	54.2	6.8	38-60
Women aged 50 and 54 in the population sample	578	52.0	2.2	50-54

Statistical methods

Conventional methods were used for calculation of mean values S.D and standard error of the mean (S.E.). Significance of differences between mean values was estimated with Student's t test (two tailed test)³¹. The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation^{31,32}. The following formula was used

$$\sqrt{P \times (1 - P) \times \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

where P_1 is the proportion of women with a certain characteristic in one of the IHD groups and n_1 the number of women in this group P_2 the proportion among those in the reference group who do not have the same IHD manifestation and n_2 the number of women free from the IHD manifestation studied in the reference group P is the pooled proportion of P_1 and P_2 according to the formula

$$P = \frac{n_1 \times P_1 + n_2 \times P_2}{n_1 + n_2}$$

If the null hypothesis could be rejected the following alternative hypothesis was accepted: a certain characteristic is more common in women with IHD than in women in the general population. Thus a one-tailed test was applied with a few exception (when the variable studied was not expected to be more common in one group than in the other according to previous studies of men or women). If a two-tailed test was used this is discussed separately in the various chapters.

When testing the hypothesis that the values of the women with IHD have the same central tendency as those of the women in the general population the following formula was used

$$P_1 = 0.5$$

$$\sqrt{0.5 \times 0.5 \times \frac{1}{n_1}}$$

where P_1 is the proportion of women in one of the IHD groups with values above the median of the population sample and n_1 the number of women in the IHD group. The alternative hypothesis was women with IHD have values higher than those of women in the general population (one tailed test).

The difference was considered statistically significant for $p < 0.05$. The data from the population study were analysed by means of a computer (IBM 360/65). The data from the women with MI were usually treated by means of a desk calculator (Hewlett Packard 9100A). The statistical analysis of stress and behavioural traits were carried out by Mr Anders Odén. Mrs Gullbritt Palm was consultant statistician and was responsible for the computer analysis of all the other variables studied.

COMMENTS

The population sample Basis of the method of selection the population sample is considered to be representative for the general population of women of the age studied. The high participation rate ensures that the results obtained in the study are representative for the same population. Telephone interview with most non-participant together with the information obtained from inpatient and outpatient records further confirms this assertion that the results obtained are representative for the general population of women in Göteborg.

Women with AP and coronary ECG. Among the women with AP and coronary ECG who participated in the population study they were representative for the women of similar age with this manifestation in the general population.

Women with MI. A *u vivo* selection in the hospital. The Migoepi believed to include almost all women born in 1913 or later who had a symptomatic MI during the period. Practically all people with symptoms of acute MI in Göteborg go directly to the emergency department of Sahlgren Hospital. If a doctor is asked to see the patient at home the doctor will immediately transfer the patient to the hospital. The condition is probably somewhat different from those in other countries, e.g. in U.K. and U.S.A. where subjects with MI more often seem to be called for at home.^{6, 91}

In order to check the completeness of the MIGOPI during 1970 the

diagnoses of the patients in departments other than those of internal medicine were studied e.g. the departments of allergology, neurology and surgery.⁵⁰ Subjects with MI born in 1903 or later were registered during this year. In all, 390 subjects were then registered and another 13 subjects (3.2 %) with an acute MI were found to have been cared for at departments other than those of internal medicine. However, no woman born in 1913 or later was among these 13 subjects with MI.⁵⁰

Interviews and when needed supplementary studies of hospital records of 4734 men in Göteborg born in 1915-1930 revealed that 1033 men with an acute MI during the years 1968-1970 had not been registered in the MI register.⁵⁰ Another previous study on the occurrence of non-hospitalized cases of suspected or definite MI revealed that only one of 161 survivors remained at home in spite of a clinical MI.⁶¹ Taken together, these observations guarantee that the MI group is almost complete with regard to the number of women with clinical MI. The low primary mortality¹⁷ and the high participation rate in the follow-up study ensured valid data on women who suffered MI without an immediately fatal outcome. At the standardized clinical controls 3 and 12 months after the MI, 38 (90 %) and 41 (98 %) of 42 survivors participated. In addition, all the 42 survivors were interviewed and examined by the author on an extra occasion.

The number of non-symptomatic MI in Göteborg is not known. None of 1462 women participating in the population study had ECG abnormalities denoting previous MI (Minnesota Codes 1-1-2) which may suggest that silent MI is infrequent in women in Göteborg.¹¹

Women with MI deaths outside hospital. MI resulting in sudden death was almost always recognized as 80 % of women who died from cardiovascular disease outside hospital in Göteborg were autopsied.⁵⁰ In addition, some women had a history of severe chest pain preceding death which strongly suggests an acute MI.^{17, 16} The number of deaths classified as MI or coronary deaths probably included all women in the ages studied who died from IHD during the period. However, as this study was not prospective, comprehensive valid data were mostly not obtained on the women who died outside hospital.

Validity of the diagnoses of MI, AP and coronary ECG. The criteria of MI in this present study are those proposed by the Swedish Heart Society and have been used previously.⁴³ There was good agreement between these criteria and the clinical impression of the physicians who treated the patients during their stay in hospital.

It is more doubtful as to what extent the criteria of AP and coronary ECG discriminated subjects with IHD. The criteria of AP according to Rose¹⁵⁵ have been widely used and they have in previous studies been found to have a high specificity and sensitivity when compared to physician diagnoses on clinical grounds.^{4, 56} They were considered to discriminate subjects with AP fairly

well in the present study¹¹ though they were probably less reliable than those of MI. The ECG criteria of IHD used in the present study¹ have previously been used in for instance Tecumseh U.S.A.⁵⁷ and in Busselton Australia.¹⁹⁶ Coexistence of AP and ECG changes denoting IHD were very uncommon in both studies as well as in the present study (Table IV).

Table IV AP and coronary ECG as single manifest stations and in combination (n). The studies in Tecumseh U.S.A.⁵⁷ Busselton Australia¹⁹⁶ and the Study of Women in Göteborg 1968-1969

	Only AP	AP + Coronary ECG	Only Coronary ECG
Tecumseh ^a	9	1	22
Busselton ^a	6	1	16
Göteborg ^b	26	3	20

^aWomen aged 40-59 ^bWomen aged 38-60

Minnesota Code 41 and 512 were the predominating ECG manifest stations in women both in Tecumseh and Busselton, while Minnesota Code 1112 was common in men. In the present study Minnesota Code 41 and/or 512 were recorded in 11th women with coronary ECG and Minnesota Code 1112 in none. It is seen that the ST depression coded as Minnesota Code 41 and the negative Q diphasic T amplitude coded as 512 (even in the absence of high R amplitude coded as 31) are not specific for IHD in women. The vast majority of the women with coronary ECG in the present study had arterial hypertension.^{11,17} It therefore is probable that coronary ECG characterized by Minnesota Code 41 and 512 is a manifestation of hypertensive disease rather than IHD in women.¹¹ However, the possibility that coronary ECG could be a pre-symptomatic manifestation of IHD in hypertensive subjects has also to be considered.¹

Cardiomyopathy of various origin has been reported to cause ECG changes¹²⁵ but is probably not a common contributor to ECG changes in women in Sweden.

Retrospective studies on the association of ECG and clinical studies. A prospective study seems to be the ideal way to obtain information about the association between different variables and IHD. Prognostic data are more valid as the data are perhaps more influenced by the variables if the study is at the time of onset of the symptom. However, even in prospective studies such as the Framingham U.S.A.⁵⁷ and in Tecumseh U.S.A.⁵⁷ the number of women who suffered IHD during long-term follow-up is still very low. The number of women who had MI was quite small. The Framingham Study comprised 2844 women aged 30-59 and the Tecumseh Study 2157 women aged 16 or

old r who we free of IHD on entry to the studi s The wid ange of the age group in the studi s makes th interpr tation of th r sults ev n mo e difficult

Th low incid nc of MI in young and middl aged women r quires v ry larg series in o ll to be able to carry out valid tatistical analysis In Göt borg with an annual incid nce of MI 0.39 p 1000 women ag d 50-54 including sudd n d ath 0.56 per 1000^{III} about 40 (in luding sudd n death about 60) women would be expect d to d velop MI within 5 years If 40000 50 y ar old wom n were in lud d in a p o pective study Thu a very large mat rial and/or a v ry long follow up period would be n cessary Th pr s nt method of studying as ocl tion b tw n diff r nt variabl s and MI is not com id r d to b id al but is probably th b t on in ord r to rec ive an answe r within a reasonabl tim

How ver when carrying out a cas control study it is n sary to be awar of it limitations Systemati differenc s b tw n th affl t d subjects and their cont ols and influenc of the dis ase studi d per s s em to be the two main limiting facto s

R f r nce group in th pr s nt study When possibl th total population sampl was u d as a ref renc group This was u ally poss ible when studying pa ametri variabl s It wa not po s bl to study non param tric variables in th s m way The women in the age strata 50 and 54 had about the same m n age as the women with IHD but it is to b emphasiz d that th ag distribution wa not th same In the women with MI and in the c with orinary ECG th wa ome accumulation in th upp r ag trata whil the age di tribution of th women with AP was similar to that of th ref nce group^{II IV} Th diff r nc in ag d str ibution betw n the wom n with MI and th eferenc group must be taken into consid ation pecially when the variabl xpe t d to chang markedly with g during th sixth decad Fo example one of the vari bles studi d wh n discuss ing the po sible influ n of str ss facto s 'last child l ft home during th last y ar' was found to be most common n wom n ag d bout 54^I When studying th str s facto s wom n ged 54 had been cho en a a r f ren e group It wa found that the l st ch ld had left hom during th last y ar more often in the e f nce group than in th MI group but th might be explained by different age distribution n the two g oup Simula ly a slightly higher pr val nc of diabet s mellitus may be xplained by the fa t that the pr val n of diabet s mellitu in e as s pld ly with g How ver when th diff ren betw cn th wom n with MI and the women in th e f ren group was a prominent as fo diabet in th pr s nt study th ag f to s could e rul d out Smoking might be expected to be l common in the wom w th MI a moking s l s s common in older women If the oppo lit s found a w th cas in the p nt study^{VII} th diff ren in

age distribution give further strength to the finding

It is difficult to have a perfect reference group in case control studies like the present one. The great advantage of the present reference group is the fact that the women in these groups are representative for the general population of women in the same way as the women with IHD are representative of the women with IHD in the same general population. The different age distribution must be taken into consideration when studying the non-parametric variables.

Statistical analyses of non-parametric variables were usually carried out on women with a manifestation of IHD and those in the reference group without this manifestation. For example, a comparison was made between women in the AP group and those free from AP in the reference group. None of the participants in the population study aged 50 or 54 who usually provided the reference group for comparisons of non-parametric variables had a history of MI. Only one of them suffered MI during the period 1968-1970. Her influence on the reference group as a whole could be neglected and she was therefore not excluded from the reference group when carrying out comparison between women with MI and women in the reference group. The comparisons of parametric variables between women with and without IHD were usually referred to the median values of the total population sample. As the women in the IHD groups could only have a minimal influence on these values in various age groups, this possible influence was neglected, and the comparisons were always referred to the median values of the total population sample including the women with AP or coronary ECG. This simplification could not bias the statistical analysis.

Influence of MI or AP per se on various variables. An acute MI may decrease the blood pressure¹ and reduce the serum lipids¹¹. Chest pain in subjects with AP may reduce the physiological activity and this in turn may have secondary effects. As AP is a common feature in subjects who suffer from acute MI¹², chest pain and its consequences may have influenced a number of variables before the MI attack. Such factors may cause difficulty when interpreting the results and have been discussed in the various chapters. Efforts have been made to rule them out as much as possible. For instance, the history of hypertension and family history of hypertension have been studied instead of the blood pressure value in women with MI. Cholesterol and triglyceride measurements have been carried out on several occasions¹¹ and mental stress was measured as exposure to certain defined stressors¹³ in addition to the woman's report of subjective experience of stress¹. In this way the influence of the MI per se is considered to be eliminated as much as possible, but the discussion is naturally limited when there is lack of precise data.

Possible influence of interobserver variation The examinations and interview in the population study were performed by the same examiners. As the women with AP were participants in the population study they were studied in exactly the same way as the other participants. The examination and interview of the women with MI were carried out by a team of physicians which makes interobserver variation probable within the MI group and between the women with MI and the population sample. However interobserver variation is not considered to be an important source of error in the present study.

Similarly the population studies of men and women were carried out by different examiners. There were also some other differences. The study of 50 year old men was carried out in 1963¹⁸² and the men were re-examined in 1967. The first study of the men was thus performed about 5 years before the study of the women. The importance of this time difference is unknown but is probably not great. Some discrepancies in method of examination have been discussed previously¹⁸³. However as a whole a similar methodology was applied to both studies. In addition both samples are representative of the same general population and this is regarded as the most important point.

Considerations on the significance of risk factor

Risk factor is a commonly accepted expression in epidemiological research. The expression is to some degree misleading as it may hint that the factor is also aetiological. A risk factor may be aetiological but is not necessarily so. It is important to emphasize that a risk factor means a detectable attribute or circumstance of an individual which is known to be statistically associated with an increased risk of developing overt morbidity but nothing more. The factor does not in itself become essential for developing disease but may be statistically associated with another factor which may be an aetiological one. The factor may be of importance only in combination with one or more other known or unknown factors. Intervention aimed at eliminating risk factors therefore does not necessarily mean a decreased risk.

Chapt II
PREVALENCE OF ISCHAEMIC HEART DISEASE IN WOMEN
IN GÖTEBORG SWEDEN

Carl Bengtsson

Abstract From a population study of middle aged women in Göteborg Sweden the prevalence and history of myocardial infarction angina pectoris and ECG changes suggestive of ischaemic heart disease. A comparison is made with men of the same ages living in the same area. The prevalence of myocardial infarction was found to be higher in men than in women while that of angina pectoris was about the same in both sexes. The prevalence of the ECG waveform common in men than in women while other ECG changes suggestive of ischaemic heart disease such as ST depression and T wave inversion were common in women as in men.

A great deal of information about ischaemic heart disease (IHD) has been obtained from mortality statistics. Population studies have also given valuable information about various manifestations of IHD. The present communication deals with the prevalence of myocardial infarction (MI) angina pectoris (AP) and electrocardiographic changes suggestive of IHD (coronary ECG) as found in a population sample of women representative of the total number of women aged 38-60 in Göteborg Sweden.

MATERIAL AND METHODS

The data were obtained from a population study of 1462 women carried out in Göteborg during 1968-1969^{1, 2}. All participants were questioned concerning a history of MI and AP. AP was defined according to Rose³. The ECG was reinterpreted according to the Minnesota Code⁴ with the Scandinavian modification⁵. Minnesota Code 11241512 (in the absence of 3:1) 6171 were defined as coronary ECG.

Further details of material and methods are given in Chapter I.

RESULTS

Prevalence of IHD in women

History of previous MI As seen from Table I there were only 2 out of 1462 women in the population study who had had a verified MI on age 46 and the other aged 60. Another 2 women had a history of severe chest pain lasting more than 30 minutes. They had not been hospitalized and their ECG did not reveal changes suggestive of MI.

Angina pectoris As shown in Table I the prevalence of AP increased with age from 0.3% in women aged 38 to 3.9% in women aged 54. As the population sample contained so few participants aged 60, conclusion concerning the prevalence in this age must be drawn with caution.

Tabl 1 Prevalence of MI AP and coronary ECG
The Study of Women in Göteborg 1968 1969

Age (years)	Subjects at risk	Subjects with MI		Subjects with AP		Subjects with coronary ECG	
	n	n	%	n	%	n	%
38	372	0	0	1	0.3	1	0.3
46	431	1	0.2	6	1.4	5	1.2
50	398	0	0	12	3.0	4	1.0
54	180	0	0	7	3.9	3	1.7
60	81	1	1.2	3	3.7	10	12.3

Coronary ECG 'Coronary ECG' was recorded in 23 women (Table 1). The prevalence increased with age. ST depressions coded as 4.1 according to the Minnesota Code were recorded in 18 women and T wave changes coded as 5.1.2 in 22 women (5.1 in 3 and 5.2 in 19). Co-existence of 4.1 and 5.1.2 was found in 17 women. In addition Minnesota Code 7.1 (left bundle branch block) was recorded in 2 of these women. No ECGs with Q and QS pattern coded as 1.1.2 or complete atrio-ventricular block coded as 8.1 were found. Minnesota Code 3.1 (high amplitude of R waves) was recorded in 5 women who also had ECG changes classified as Minnesota Code 4.1 and 5.1.

Fig 1 shows the co-existence of AP and coronary ECG. Only 3 women were classified as having both AP and coronary ECG.

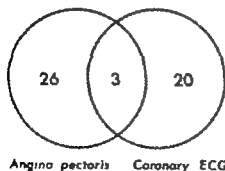


Fig 1 Co-existence of
AP and coronary ECG
The Study of Women in
Göteborg 1968 1969

Prevalence of IHD among the non-attenders. Information about heart disease was obtained by interview from 116 of 128 women (91 %) who refused to attend the population study and from records in another

3 women who had visited the medical gynaecological or psychiatric service during the previous three years. In this way information was obtained from 93 % of those who refused to attend. None of them had had a verified MI and none complained of chest pain.

Women who had died in 1950 or later and who would have been called for the population study if still alive were studied by means of their death certificates. Six out of a total of 56 had died from disease of the heart according to the death certificates.¹ One of these women had died from congenital heart disease, two from rheumatic heart disease, two from IHD and one from periparturient heart. The diagnosis of the first mentioned 5 women were based upon autopsy findings.

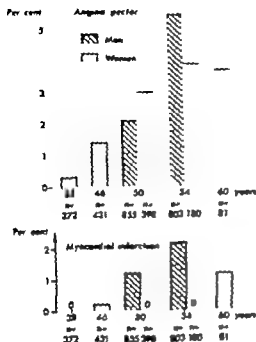
Prevalence of IHD as compared to other heart diseases in women. It was not intended to study in detail the prevalence of various congenital and rheumatic heart diseases in the population sample. However, some information was obtained from history and physical examination. The prevalence of the heart disorders was low (Table II). Four women had previously been diagnosed as valvular heart diseases. In 8 women murmurs were found making heart disease probable. The prevalence of IHD was higher than the prevalence of the other heart diseases. A reported above 2 women had died from IHD and from rheumatic heart disease in 1950 among those who would have been included in the sample had they still been alive.

Table II Prevalence of heart disorders
The Study of Women in Göteborg 1968-1969

	n	%
Verified myocardial infarction	2	0.1
Angina pectoris	29	2.0
Coronary ECG	23	1.6
Verified valvular heart disease prior to the examination	4	0.3
Heart murmurs suggestive of congenital or rheumatic heart disease previously unknown	8	0.6

Comparison between the prevalence of MI, AP and coronary ECG in men and women. A comparison between the prevalence of MI and AP in men and women from Göteborg is made in Fig. 2. A population study of men born in 1913 had been performed in 1963 when the men were 50 years old.¹⁸² The men were examined 4 years later at the age of 54.¹⁸³ The prevalence of AP was about the same in the two sexes at the age of 50 and 54 but the prevalence of MI was much higher in the men.

Fig 2 Prevalence of AP and MI in men and women in Göteborg. The Study of Men Born in 1913^{107 108} and the Study of Women 1968-1969



The prevalence of ECG changes suggestive of IHD could be compared between 54 year old men and women (Table III). Q waves (Minnesota Code 1:1 2) were recorded in 1.6% of the men but in none of the women while ST depressions (4:1) and inverted T waves (5:1 2) were as common in the women as in the men.

Table III Prevalence of ECG changes suggestive of IHD in 54 year old men (n=793) and 54 year old women (n=180) in Göteborg. The Study of Men Born in 1913^{107 108} and the Study of Women 1968-1969

ECG category Minnesota Code	Men		Women	
	n	%	n	%
1:1 2	13	1.6	0	0
4:1	12	1.5	3	1.7
5:1 2	16	2.0	3	1.7
6:1	0	0	0	0
7:1	2	0.3	0	0

DISCUSSION

Acherson, Epstein and Traub⁸⁸ have reviewed reports on mortality in IHD, prevalence and incidence of IHD and sex ratio in different countries. The sex ratio is different in different races and countries. However, the constant

finding of a higher prevalence of MI in middle aged men than in middle aged women in the industrial countries agrees with the results of the present study in Göteborg

Population studies have given limited data concerning IHD in women. Because of the differences in criteria methods of sampling age grouping and presentation the results of the various studies are seldom comparable. However in Table IV an effort is made to compare the data obtained in Göteborg with those from some previous investigations in men and women viz the population studies in Framingham U.S.A.⁴² Tecumseh U.S.A.⁵⁷ and Glostrup Denmark.⁷² The wide span of the age group in the presentations of data from Framingham and Tecumseh makes comparison with the Göteborg data difficult. The Scandinavian figure for MI is about the same as the corresponding U.S.A. figures. AP seems to be more frequently reported in Scandinavia both in men and women. This difference may be explained by differences in criteria and in the manner of presentation of data.

Table IV Prevalence of MI and AP (%) in men (M) and women (F) in the Framingham⁴² Tecumseh⁵⁷ Göteborg and Glostrup⁷² studies

Framingham				Göteborg				Glostrup			
Age (year)	MI		AP	Age (year)	MI		AP	MI	AP	MI	AP
	M	F			M	F		M	F	M	F
30-44	0.2	0	0.2	0.4	38	0	0.3				
45-62	1.6	0.1	1.9	1.2	46	0.2	1.4				
	Tecumseh			50	1.4	0	2.1	3.0	0.9	0.4	4.2
40-59	2.5	0	2.0	1.2	54	2.0	5.5	3.9			
				60		1.2		3.7			

It is of special interest to compare the prevalence data from Göteborg with those from Glostrup Denmark as 50 year old men and women were studied in both places. There were no differences in the prevalence of MI or ex difference of MI (Table IV). The Glostrup study revealed a moderate ex difference of AP which was not found in Göteborg. The moderate absolute ex difference in the prevalence of AP is in agreement with the result from other studies.^{51, 57, 70} Thus the prevalence of AP is about the same in men as in women but there must be some factors making men more liable to MI than women as will be discussed further in Chapter XIV.

ECG changes classified according to Codina^{11, 2} were more common in the men than in the women while there was no sex difference concerning ST depressions (Minnesota Cod 4.1) and T wave inversion (5.1). This agrees

closely with previous observations in Tecumseh U.S.A.⁵⁷ and in Bussleton Australia.¹⁴

It is obvious from previous studies and from the present study that all manifestations of IHD increase with age. Conclusion about the prevalence figure concerning the 60 year old participants of the present population study must be drawn with caution as there were few women in this age group.

It is obvious that the number of women who had died from IHD was small as shown when the causes of death were studied in those who would have participated in the population study if still alive. The low prevalence of MI in women in Göteborg was thus not due to an early death from this cause.

It can be debated whether the criteria used for the various manifestations of IHD really detected those with IHD. Only 3 women were considered to have both AP and coronary ECG while most of them had only one of these manifestations (Fig 1). This may suggest that the criteria are not sufficiently specific. Similar observations have been made in other parts of the world.¹ The standardised questions for AP were found to have a high specificity and sensitivity when compared with physicians' diagnosis on clinical grounds as shown in two previous studies of men.^{81, 155} Even though the sensitivity and specificity in women are not necessarily similar it seems probable that the standardised questions will discriminate subjects with AP fairly well.

It is more doubtful whether the coronary ECG as defined in the present study really discriminated women with IHD. It was found that out of 23 women with coronary ECG 11 were on antihypertensive treatment. Another 2 were on diuretic for other reasons. Of the 10 women who were not on antihypertensive treatment or diuretics 8 had a systolic blood pressure above the median for the population sample.⁷ The ECG changes of the women with coronary ECG were therefore suspected of being a manifestation of hypertension rather than IHD.¹

Due to the high participation rate and the collection of information about the non-attenders in whom no overrepresentation of IHD was found the results obtained from the present population study concerning the prevalence of IHD are considered to be a valid estimate of the criteria for the various manifestations.

IHD in women, especially MI, is rare. It should be noted, however, that the present population study revealed a higher prevalence of IHD than of valvular heart disease in women of the age studied. Although less frequent than in middle aged men IHD in middle aged women is frequent enough to be of importance.

Chapt r III
INCIDENCE OF MYOCARDIAL INFARCTION IN WOMEN
IN GÖTEBORG SWEDEN

Carl Bengtsson

Abstract The annual incidence of myocardial infarction in Göteborg Sweden increased from 0.03 per 1000 women aged 35-39 to 0.39 in women aged 50-54 including sudden death from 0.05 to 0.56 per 1000. The incidence was about 8 times higher in men than in women.

Information about the incidence of myocardial infarction (MI) in men has been obtained from population studies. Less is known about the incidence of MI in women except that it is low. If it is to be studied in a population sample of young or middle aged women the sample must be very large or the follow up must continue for a long time if reliable information is to be obtained. During a mean follow up period of about 4 years only 2 of 1462 women who participated in a population study in Göteborg Sweden in 1968-1969^{1, 15} suffered MI in order to obtain more valid information the incidence of MI was studied in the total number of women living in Göteborg who were born in 1913 or later.

MATERIAL AND METHODS

Data were obtained from a MI register which included all subjects (men and women) known to have had an acute attack of MI during the years 1968-1970 who lived in Göteborg and were born in 1913 or later^{1, 50}. A detailed report on the incidence of MI and IHD death in men and women is given by Elmfrid et al.⁵⁰

The population at risk consisted of all individuals living in Göteborg who were born in 1913 or later^{171, 172, 173}. The total number of subjects in different age groups who had a MI during the years 1968-1969 and 1970 e.g. those aged 50-54 at the beginning of the years was added up. Similarly the total number of individuals at risk in the same age groups throughout the 3 years was added up and the mean annual number of subjects with MI per 1000 individuals was calculated. The women who were 55 years or older at the beginning of the year in which they had their MI or died were not included when calculating the incidence figure.

Deaths outside the hospital were classified as MI when autopsy revealed a fresh MI as well as when a severe chest pain preceded death^I. Two women were classified as death outside the hospital although they were in hospital for a longer time than heart disease^{IV}.

Further details of material and methods are given in Chapter I.

RESULTS

Forty seven women with MI were survivors on arrival in hospital while 10 women died from an acute attack of MI outside hospital. The death of another 4 women classified as coronary deaths was probably caused by an IHD attack. Those aged 54 or younger are presented in Table I. Table I also shows the annual incidence of MI and death outside hospital from a probable IHD attack in women aged 34 or younger. The annual incidence of MI in women increased from 3 per 100000 in the age range 35-39 years to 39 per 100000 in the age range 50-54. If non accidental unexpected sudden death is included the incidence became 5 and 56 per 100000 respectively. Of 12 women aged 54 or younger who died suddenly 9 were considered to have died from MI and 3 were classified as coronary deaths.

Table I Annual incidence of MI and sudden death (non accidental unexpected death within a few hours) per 1000 women in Göteborg born in 1913 or later (age at the beginning of the year is recorded the incidence figures in brackets include sudden deaths)

Age group	Women hospitalized because of MI 1968-1970	Sudden death outside hospital from probable IHD 1968-1970			Women at risk 1968-1970 ^a	Annual incidence
		MI	Coronary death	Total		
35-39	1	1	0	1	37418	0.03(0.05)
40-44	3	1	0	1	42263	0.07(0.09)
45-49	11	1	1		49594	0.22(0.26)
50-54	18	6		8	46131	0.39(0.56)

^aThose at risk each year are totaled

Comparison between the incidence of MI in men and women

A comparison between the incidence of MI in men³⁰ and women revealed that men predominated in all age groups (Fig 1). The incidence was about 8 times higher in men. Non accidental unexpected sudden deaths outside hospital were also much more frequent in men.

Sex ratio

♂/♀

14-

12-

10-

8-

6-

2-

0-

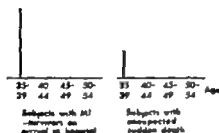


Fig 1 Sex ratio for MI (survivors on arrival in hospital) and unexpected non-identical sudden death. The year in incidence in Göteborg

DISCUSSION

The incidence figures in the present report were obtained by studying the incidence of MI in the total general population of subjects aged 54 or younger in Göteborg. In Table II a comparison is made with the incidence found in a population sample of women in Framingham U.S.A.¹² and in the total population of women in Kristiansund Norway⁷⁰ and Helsinki Finland.¹⁵⁴ The

Table II Incidence of MI (sudden death not included) per 1000 women per year in Framingham¹² Kristiansund⁷⁰ Helsinki¹⁵⁴ and Göteborg

Age (year)	Incidence of MI per 1000 per year		
	Framingham ^a	Göteborg ^b	
30-44	0	0.05	
	Kristiansund	Göteborg ^b	Helsinki
30-34	0	0	0
35-39	0	0.03	0
40-44	0	0.07	0.06
45-49	0	0.22	0.40
50-54	0.6	0.39	0.97

^aAge entry to the study. Figure calculated from 4 year incidence at the beginning of the year. Age at onset of the symptom.

^bAge at

incidence of MI in women seems to be similar in Göteborg Framingham and Kristiansund. However, it is to be noted that the women at risk were few in both Framingham and Kristiansund. The incidence was higher in women aged 45 or older in Helsinki than in Göteborg. The incidence was similarly higher in men in Helsinki¹⁵⁴ than in men in Göteborg⁵⁰. The number of sudden deaths was also larger in Helsinki. Göteborg and Helsinki have about the same number of inhabitants and the way of life seems to be similar in the two cities. The difference in the incidence of MI and sudden death between Göteborg and Helsinki is, therefore, surprising and cannot as yet be explained.

In agreement with previous studies in other places^{54, 164, 188} the incidence of MI and sudden death was higher in men than in women in Göteborg.

An important question is whether all new cases of MI in the area were registered. In the Göteborg area there is only one hospital for acute heart diseases which simplifies the registration of all hospitalized cases. Almost all subjects with MI in Göteborg are taken to hospital.⁴¹ The number of unregistered cases with symptomatic MI was less than 10%^{1, 90}.

The occurrence of "silent MI" in the area is not known. However, none of the women participating in the population study in Göteborg¹⁵ demonstrated ECG abnormalities suggestive of a previous MI (Minnesota Code 1:1 or 1:2^{II}). This finding may suggest that silent MI is infrequent in women in Göteborg. Although the occurrence of silent MI is probably low, it cannot be neglected. The incidence rates thus do not include individuals with non-fatal silent MI resulting in sudden death, however, it is almost always recognized as about 80% of women who die from cardiovascular disease outside hospital in Göteborg are autopsied.⁹⁰ Eleven of 14 women (79%) who had died suddenly outside hospital in the present study were autopsied. If autopsy has not been performed, severe precordial pain preceding death strongly suggests an acute MI. It has thus been reported that 20 of 23 patients who had sudden death preceded by central chest pain had a fresh coronary artery thrombosis and/or fresh MI on autopsy.¹¹⁶

CLINICAL CHARACTERISTICS OF YOUNG WOMEN
WITH MYOCARDIAL INFARCTION

Gull Bengtsson and Gösta Tibblin

Abstract A history of previous myocardial infarction and angina pectoris was more common in women with an acute attack of myocardial infarction than in the general population of women as was symptom of angina pectoris and intermittent claudication. Chronic bronchitis, gallstone disease and renal tone diseases were also overrepresented. The women with myocardial infarction were usually inactive when taken ill. About one third of them complained of breathlessness and/or disturbance of consciousness associated with the attack. Most women with myocardial infarction had symptoms such as nausea, vomiting and cold perspiration. The infarction area was localised with about the same frequency to the anterior and posterior wall of the heart. In about half of the women the maximum registered temperature exceeded 38.0°C . The primary mortality of the women who were hospitalised was rather low (9%). The overall mortality among those who were survivors on arrival in hospital and those who died outside hospital during the first few hours or days was 30%.

Myocardial infarction (MI) in women is rare below the age of 60 compared to men of the same age^{II,III}. Thus there are few reports on the clinical picture of MI in young and middle aged women. Previous reports^{12,19,165} have usually dealt with clinical risks which cannot be considered as representative for the total number of women with MI in a special geographical area. Some other previous studies have included women with other manifestation of ischaemic heart disease (IHD) than MI^{9,30,39}.

The present report deals with the total number of MI in women born in 1913 or later who lived in the defined area of Göteborg, Sweden during the years 1968-1970. Some characteristics and a description of clinical features in these women are given.

MATERIAL

All subjects with an acute attack of MI who were born in 1913 or later and who live in Göteborg have been registered in the list of January 1968 at the Post MI Clinic^{I,30}. Death certificates and autopsy reports of persons born in 1913 or later and who lived in the same area have been studied in order to include non-hospitalised subjects dying from MI. The present material was collected during the years 1968-1970. One woman aged 23 with systemic lupus erythematosus was excluded since her MI was considered to be a complication of the primary underlying disease²³. The series comprised 47 women who were survivors on arrival in hospital. Four of them died before being interviewed by members of the Post MI Clinic staff. Another 10 women who were not hospitalised for heart disease died suddenly from MI^I. The deaths of another

4 women who were not known to have a history of severe heart pain preceding death and who had coronary atherosclerosis but no fresh MI area on autopsy were classified as coronary deaths¹

METHODS

In connection with the acute attack of MI the women were interviewed in a standardized way by one of five physicians from the Post MI Clinic. Angina pectoris (AP) and intermittent claudication were defined as proposed by Rose¹⁵⁵. Chronic bronchitis was defined as coughing and sputum in the morning and during the day or night for at least three months a year¹²³. A history of gall stones or renal stones was obtained by means of a questionnaire. The examinations during the hospitalization period were performed in a standardized way in the medical wards. Eighteen women (38 %) were taken to the coronary care unit.

Routinely the rectal temperature was recorded twice daily. ECG and determinations of SGOT were performed on each of the first three days. A leucocyte count was done during the first or second day. Laboratory analysis, ECG recording and X-ray were performed according to the routine methods used at Sahlgrenska Hospital Göteborg. SGOT was determined as Carmen units before July 1st 1970 and as U/l after this date. Two Carmen units correspond roughly to one U/l up to the level of 100 Carmen units (50 U/l). Values ≥ 40 Carmen units or ≥ 17 U/l were considered as pathological. X-ray was performed with the patient standing. The roentgenological heart size was calculated from frontal and lateral films⁹⁰.

Statistical methods. The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation.

Further details of material and methods are given in Chapter I.

RESULTS

Age

The ages of the women who were hospitalized for MI are shown in Fig. 1. The youngest woman was 37 years old when she suffered MI. The ages of those who were later found to have had a MI and coronary death are shown in Table I.

History of chest pain and autopsy findings in the women who died.

Table I shows the prevalence of severe heart pain and autopsy findings in women who died suddenly and who were not hospitalized for suspected MI. Twelve of the women died outside hospital while 26 were institutionalized for reasons other than heart disease (bacterial and stroke respectively).

Fig 1 Age distribution of women with MI in Göteborg during the years 1968-1970 born in 1913 or later (survivors on arrival in hospital)

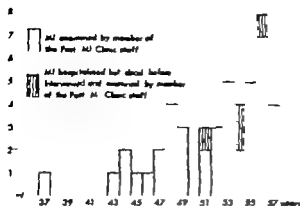


Table I Age pre-onset of severe chest pain preceding death and autopsy findings in women who died suddenly and unexpectedly and who had not been hospitalized because of suspected MI

Subject No	Age (year)	Chest pain	Autopsy	Free MI area on autopsy	Severe coronary atherosclerosis on autopsy
Classified as MI ¹					
1	40	?	Yes	+	+
2	42	+ ^a	Yes	+	+
3	48	+	No		
4	51	+	Yes		+
5	51	+	No		
6	51	+	Yes	+	+
7	53	+	Yes	+	+
8	54	+	No		
9	54	+ ^a	Yes	+	?
10	56		Yes	+	+

Classified as coronary death¹

1	48		Yes		+
2	54	?	Yes		+
3	55		Yes		+
4	56		Yes		+

^a Acute heart failure predominant symptom preceding death.

Table II History or prevalence of some diseases in 47 women with MI survivors on arrival in hospital compared to a reference group (578 participants in a population study of women in the age strata 50 and 54)

	Women with MI %	Reference group %	Significance of difference
History of MI	12.8	0	$p < 0.001$
History of severe chest pain ≥ 30 minutes	23.4	0.5	$p < 0.001$
Angina pectoris	46.8	3.3	$p < 0.001$
Chest pain on time	70.2	4.4	$p < 0.001$
Intermittent claudication	11.1 ^a	1.7	$p < 0.001$
Chronic bronchitis	20.5 ^b	2.9	$p < 0.001$
History suggesting gallstone disease	35.6 ^a	21.8	$p < 0.05$
History suggesting renal stone disease	13.6 ^b	5.0	$p < 0.001$

^aData from 2 women missing ^bData from 3 women missing

History of previous disease

In Table II the women with MI who were survivors on arrival in hospital, are compared with regard to history or prevalence of some previous diseases with a representative sample of women from the same general population in the age strata 50 and 54.^{1,15} The women of this reference group had a mean age similar to that of the women with MI.¹ Six of the women in the MI group (13%) had a history of previous MI and almost half of them reported symptoms of AP. Symptoms of intermittent claudication and chronic bronchitis and a history of gallstone and renal stone were also more common in the MI group than in the reference group (Table II). Information about history of MI and AP was obtained for 7 of 10 women who had died from a MI attack without being hospitalized for heart disease. Five of the 7 women had a history of AP while none of them had a history of MI.

Activity when taken ill

Information about activity when taken ill was obtained from all the 47 survivors. Thirty-nine (83%) stated that they were at rest when taken ill, one (2%) that she was undressed and seven (15%) that they were physically active. Four of them were under physical treatment. Valid information was obtained from only a few of those who died outside hospital.

Time when taken ill

The attack of MI occurred frequently during the day as during the night.

Information was obtained from all the 47 women. Nineteen (40 %) suffered the attack during the day 18 (38 %) during the night 6 women (13 %) in the morning and 3 women (4 %) in the evening while 2 women with pain lasting for more than 24 hours on arrival in hospital could not say when the pain began. Valid information was obtained concerning 9 of those who died outside hospital or who were in intensive care for reasons other than suspected MI. The attack started during the day in 4 of them, in the evening in 2 and during the night in 3. The attack of survivors tended to occur more often during the autumn and winter than during the spring and summer while the death were evenly distributed during the year (Fig 2).



Fig 2 Seasonal variation of MI in women. White columns: MI survivors on arrival in hospital; dark column: MI deaths; striped column: coronary; hatched column: atherosclerosis.

Symptoms associated with the acute attack

Information was obtained only about those who were survivors on arrival in hospital.

Pain. Information was obtained from 46 women. Pain was noted in all of them. In 9 women (20 %) the pain was localised to the sternum only in 2 women (4 %) to the sternum and neck and/or jaw in 26 (57 %) to the sternum and arm and/or shoulder and in 9 (20 %) to the sternum as well as to the neck and/or jaw and arm and/or shoulder. Information about pain was not obtained in one woman whose main initial symptom was sudden loss of consciousness.

Bradycardia. Information was obtained from 43 women. Of the 30 (67 %) had had no difficulty in breathing while 15 (33 %) complained of shortness of breath.

Disturbance of consciousness. Information was obtained from 43 women. Thirty of the (70 %) had been fully conscious at the time 8 (19 %) complained of having had a feeling that everything was dim for a moment and 5 (12 %) had been unconscious for some time.

Autonomic nervous system disturbance. Information was obtained from 44 women. Nine of these (20 %) did not complain of either nausea or vomiting or cold perspiration. Four women (9 %) complained of nausea 2 women (5 %) vomited 7 (16 %) noted cold perspiration and another 22 women (50 %)

noted cold perspiration and at the same time either felt nauseated or vomited

Signs noted during the period of hospitalization

Signs of heart failure Signs of left ventricular insufficiency were reported in 14 out of 47 women (30 %) either by physical examination or X ray or both. Pulmonary rales were noted in 9 women (19 %). Radiological signs of congestion were noted in 9 (29 %) of 31 women who had had chest X rays at the time of the acute attack. In one woman manifest pulmonary oedema occurred.

Arrhythmia In 30 of 47 women (64 %) some kind of arrhythmia was recorded, mostly supraventricular tachycardia and/or supraventricular extrasystolic beats (11 subjects) and ventricular tachycardia and/or ventricular extrasystolic beats (15 subjects). Atrioventricular block was noted in 5 subjects. Ventricular fibrillation that could be regularized occurred in two women. One of them recovered and was able to leave the hospital as a convalescent. The other became unconscious in association with the attack of ventricular fibrillation and died a few days later.

Body temperature and laboratory data

Maximum noted values for rectal temperature and some laboratory data are given in Table III. Twenty six out of 47 women (55 %) had a maximum temperature $\geq 38.0^{\circ}\text{C}$. Leucocyte $\geq 10,000$ were found in 26 women (55 %). In all but 5 subjects pathological SGOT values were recorded.

Table III. Number of subjects within different intervals of rectal temperature and some laboratory values in women with MI (maximum noted values are reported).

Temperature ($^{\circ}\text{C}$)				
< 38.0	38.0-38.9	39.0-39.9	≥ 40.0	
21	20	5	1	
Leucocyte # (per mm^3)				
< 10000	10000-14900	15000-19900	≥ 20000	
1	14	9	3	
SGOT (Carmen units)				
< 40	40-99	100-199	200-499	≥ 500
2	13	8	9	2
SGOT (U/l)				
< 20	20-48	50-99	100-250	≥ 250
3	5	3	2	0

Localization of the infarction are

As judged from the ECG the anterior wall was affected in 23 subjects and the posterior wall in 19 subjects (Table IV). Q waves were recorded in 32 subjects (68 %).

Table IV Localization of the infarction area according to ECG

Localization	n	%
Anterior wall	15	32
Lateral wall	2	4
Posterior wall + septum	6	13
Posterior + lateral wall	13	28
Anterior + lateral wall	8	17
Uncertain localization	3	6
Total	47	100

Heart volume

Chest X-ray was performed on 37 women before leaving the hospital. The relative heart volume (ml/m^2 body surface) was found to be < 400 which is regarded as a normal value in 19 women (51 %), 400-499 in 12 (32 %) and ≥ 500 in 6 (16 %).

Outcome of the acute attack

Four of 47 women who were hospitalized for MI (9 %) died during the period of hospitalization. Including those who died outside the hospital or while hospitalized for reasons other than suspected MI, 14 of 57 women (25 %) died during the acute attack. If these classified as coronary death, we included the primary mortality it found to be 30 % (18 of 61 women).

DISCUSSION

Studies of young and middle aged women with MI are few and usually performed retrospectively on selected series. The present series comprise almost all women with MI during a defined period of time¹ and the subjects were not examined retrospectively but in connection with the acute attack. A plan for the investigation existed before the subjects fell ill, therefore few missing data among the survivors. The clinical characteristics of the women with MI in the present study are therefore considered to be representative for women surviving an acute attack of MI. Limited information was obtained

concerning the women who died outside hospital

A history of a previous attack of MI was common being recorded in 13 % of the hospitalized. This is less than has been reported earlier. Thus Blackman and Kologlu¹⁹ reported a previous history of MI in 33 % of women under 50 years of age and Bailly and Beaven¹³ in 2 % in a series of women where no age limit was fixed. However, these studies were performed on selected hospital series.

AP was noted in almost half of the women which is in agreement with previous reports. It was also common in those who died from MI outside hospital. Shochet¹⁶ reported a history of AP in 20 % of women with MI aged 50 or younger and Blackman and Kologlu¹⁹ in 40 % of women under 50 years of age. A similar prevalence of AP (45 %) was also noted in men with MI in Göteborg.²⁰

A history of other chest pain noted findings as AP was reported in another 11 women in the present series. This may well be in agreement with the notion that AP is often atypical in women.⁹ It is possible that atypical chest pain may be a manifestation of IHD.

It has also been stated that MI is often atypical in women²¹ but this was not confirmed in the present study. Intermittent claudication, another manifestation of atherosclerotic disease, was also overrepresented in the present MI group. It must be born in mind that the present data were obtained in association with an acute attack of MI. It is possible that the subjects are more aware of chest pain etc. and therefore report it more often than those participating in population study but this cannot explain the great difference between the two groups.

Chronic bronchitis was common in the women with MI which may be explained by a longer representation of smoking in subjects with chronic bronchitis as well as in subjects with MI.²² In agreement with previous observations on men in Göteborg²⁰ a history of gall stones or renal stones was more common in the women with MI than in the reference group.

The acute attack usually occurred while the women were at rest and in the night as often as in the daytime. The risk of having MI while undressed and physical exertion seemed to be small. Similar observations on MI survival and sudden death have been reported from Helsinki, Finland,²³ and on medically unattended deaths from Stockholm, Sweden.²⁴

Complications following an attack seemed to occur during the summer. The material is too small to allow conclusions concerning seasonal variation but the tendency found agrees with the tendency in studies of women elsewhere.^{25, 26}

Symptoms such as nausea, vomiting and cold perspiration were very common occurrences in more than half of the women.

About half of the women complained of breathlessness and/or disturbance of consciousness. Signs of pulmonary congestion noted on physical or

X ray examination were common. The heart volume seemed to be increased in some of the women. Most of the women had some kind of arrhythmia. Supraventricular or ventricular tachycardia and/or supraventricular or ventricular beat were predominant manifestations. As most of the women had not been taken to the coronary care unit minor episodes of arrhythmia were probably often not recorded. Temperature and laboratory data usually deviated moderately from normal values. The results are essentially similar to those in a previous hospital series.¹⁹

The anterior and posterior wall of the heart seemed to be affected with the same frequency which is in agreement with autopsy findings in a study of men and women ≤ 45 years of age in Finland.¹²⁰

The mortality associated with the hospitalization for the acute attack was rather low in the present material (9% of those hospitalized). Altogether 30% of the women with MI or other coronary death died within the first few hours or days after the symptoms appeared.

BLOOD PRESSURE IN A POPULATION SAMPLE OF WOMEN AND
IN WOMEN WITH ISCHAEMIC HEART DISEASE

Calle Bengtsson

Abstract Women with myocardial infarction had hypertension were on antihypertensive treatment and had a family history of hypertension significantly more often than women in the general population. The distribution of blood pressure values in women with angina pectoris did not differ significantly from that in the general population of women. Hypertensive were significantly over-represented among women with ECG changes suggestive of ischaemic heart disease. This might be interpreted like as a sign that coronary ECG as defined is often a pre-symptomatic manifestation of ischaemic heart disease in hypertensives or that these ECG changes are not specific for ischaemic heart disease.

Several communications have reported an association between high blood pressure (BP) and ischaemic heart disease (IHD) in men^{1,2,3,4,5,6}. There seems also to be an association in women although the literature here is sparse and controversial.

The purpose of the present chapter is to describe the arterial BP in a representative population sample of women and in women with IHD. Special reference is given to the question whether women with manifestations of IHD differ from women in the general population.

MATERIAL

Reference group A population study of women in Göteborg was performed in 1968-1969¹. Women in the age strata 38-46, 50-54 and 60+ were examined. 1462 women altogether (participation rate 90.1%). The total number of women participating in the population study provided the reference group when comparing parameters/variables in this chapter. When looking for differences in frequency of non-parametric variables between groups of women with IHD and women in the general population, those aged 50 and 54 (n = 378) provided the reference group. They had about the same mean age as the women with IHD.

Women with angina pectoris Twenty-nine women in the population study reported history of angina pectoris (AP) as defined according to Rose⁷.

Women with coronary ECG ECG changes implicating Minnesota Codes 112, 41, 512 (in the absence of 31), 61, 71^{8,9} were defined as coronary ECG and recorded in 23 women participating in the population study.

Women with myocardial infarction All people in Göteborg born in 1913 or later who had an acute attack of MI have been registered since the 1st of January 1968 at the Post MI Clinic in Göteborg¹. Forty-seven

women who were survivors on arrival in hospital were registered during the years 1968-1970. Of these 4 women died during the period of hospitalization and one soon afterwards. Forty-two survivors were admitted to clinical control 3 and 12 months after the MI attack.^{1,51} Another 14 women who were not hospitalized for suspected MI died from a probable IHD attack during the period 12 outside hospital and 2 during a period of hospitalization for reasons other than suspected MI.^{1V} Ten of these women were classified as MIs.¹

METHODS

BP measurements in the population study. The participants of the population study were examined according to a special schedule.¹⁵ Physical examination including determination of BP was performed about half an hour after their arrival at the place of examination. All the BP determinations were performed by the author. The sphygmomanometer was used on a regular basis. BP was measured between 07.30-10.30 a.m. in a fasting subject in a quiet room. The technique of BP determination was that recommended by WHO⁷ which had also been used in the previous examination of men in Göteborg.¹⁶ After about 5 minutes' conversation with the woman who was sitting comfortably in a chair a 25 x 12 mm cuff with nylon hooklet binding was applied firmly and evenly to the right arm. The lower edge of the cuff was about 2 cm above the antecubital pulse. The cuff was quickly inflated to 200 mm Hg and the stethoscope was applied to the edge of the cuff. The cuff pressure was lowered at a rate of about 2 mm Hg per pulse beat and the point at which the pulse beat was first audible was recorded. Diastolic pressure was defined as the point of muffling of sound (phase 4) and the point of disappearance (phase 5). When the systolic sound was audible immediately after inflation the cuff pressure was allowed to fall to zero and a fresh inflation to 250 mm Hg was performed. The BP was read to the nearest 2 mm Hg. Readings were performed in the seated position in all individuals and in the supine and standing positions (immediately after resting) in all but the first 33 women studied.

Determination of BP in the women with MI. BP was determined 3 months and 1 year after the attack of MI. The determinations were made in the morning and with the same technique as described above. BP was determined in the supine and standing positions by one of five physicians from the Post-MI Clinic.

History of arterial hypertension. All participants both those attending the population study and those with MI were interviewed concerning history of arterial hypertension. The antihypertensive treatment was recorded separately. Information on the who had died was obtained from records and medical legal reports.

Family history of arterial hypertension. Family history of

arterial hypertension was obtained in the same way

Electrocardiographic examination The ECGs were taken in a standardized way¹ and read according to the Minnesota Code¹² with its Scandinavian modification⁹

Statistical methods

Conventional statistical methods were used to calculate mean values and standard deviation (S D) The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation

Further details of material and methods are given in Chapter I

RESULTS

BP in a population sample of women

The mean values of BP at different ages are given in Table I In the seated and upin positions both phase 4 and phase 5 of the diastolic BP are given and in the standing position phase 4 only All individuals in whom BP had been determined are included even those on antihypertensive treatment at the time of the examination Decentile limits of BP in the seated position are given in Fig 1 Distributions are given in Figs 2 3 The number of women on antihypertensive treatment is also shown in these figures and together with the type of treatment in Table II Saluretic diuretics were the antihypertensive agents most commonly used

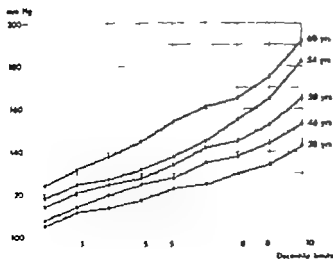


Fig 1 Decentile limits of systolic BP in the seated position The Study of Women in Göteborg 1968 1969

Table I Mean and S D of BP (mm Hg) in the seated supin and standing positions

The Study of Women in Göteborg 1968-1969

Age	Seated			Supin			Standing		
	n	Mean	S D	n	Mean	S D	n	Mean	S D
Systolic BP									
38	372	123	11.5	362	124	14.5	362	128	16.1
46	431	131	19.5	420	133	21.2	420	132	20.5
50	397	138	21.8	385	140	22.7	384	137	22.5
54	180	143	24.1	180	145	23.9	179	141	22.5
60	81	154	27.5	81	158	27.3	81	152	27.2
Diastolic BP phase 4									
38	372	81	9.2	362	80	10.7	362	88	9.3
46	431	85	10.9	420	84	10.2	420	90	10.6
50	397	88	10.6	385	87	11.4	384	93	11.4
54	180	89	12.1	180	89	11.5	179	95	12.3
60	81	92	12.3	81	93	12.3	81	96	13.5
Diastolic BP phase 5									
38	372	79	9.2	362	78	9.6			
46	431	82	10.0	419	82	10.2			
50	379	86	10.6	384	85	10.7			
54	180	87	12.0	180	86	11.5			
60	81	89	12.4	81	90	12.4			

Table II Number on antihypertensive treatment
The Study of Women in Göteborg 1968-1969

Age	Salt tablets		Other than diuretic		Salt tablets and other		Total	
	n	%	n	%	n	%	n	%
38	3	0.8	1	0.3	0	0	4	1.1
46	5	1.2	4	0.9	5	1.2	14	3.2
50	13	3.3	1	0.3	4	1.0	18	4
54	9	5.0	1	0.6	9	5.0	19	10.6
60	11	13.6	0	0	2	2.5	13	16.0

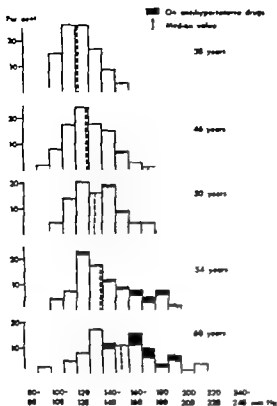


Fig 2 Distribution of systolic BP in the seated position The Study of Women in Göteborg 1968-1969

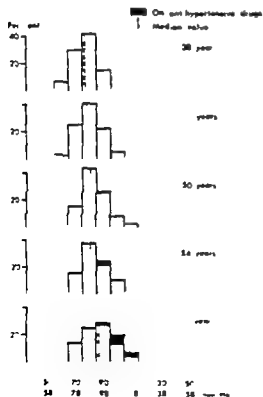


Fig 3 Distribution of diastolic BP in the seated position The Study of Women in Göteborg 1968-1969

BP in non attenders

Information about the BP on at least one occasion during the previous three years was obtained in 95 (74 %) of the 128 referents by studying records from inpatient and outpatient clinics and by interviewing the women. Treatment for hypertension was as common in the referents from whom information had been obtained as in the attenders except for the women aged 54 in whom a greater percentage of referents than attenders were receiving treatment (Table III). Two of the referents had been told that their BP was high but did not know the value and 37 of them had been told that their BP was normal but did not know the value. Fifty-six women (44 %) had been told the value of their BP. No information was obtained from the records. The mean systolic BP was somewhat higher in these referents than in the attenders (Table IV).

Table III Antihypertensive treatment in referents from whom information had been obtained (n = 95) as compared to the attenders (n = 1462)

Age (years)	Referent n	Referent %	Attender %
38	0	0	1.1
46	1	2.9	3.2
50	2	7.1	4.5
54	6	33.3	10.6
60	2	14.3	16.0

Table IV Systolic BP (mm Hg) in referents in whom the BP had been recorded during the previous 3 years

Age (years)	n	Mean	S.D.
38	13	131	15.3
46	15	139	16.0
50	16	140	24.4
54	8	171	20.8
60	5	155	21.2

BP in women with AP and coronary ECG in the population sample

Angina pectoris In Table V women with AP are placed according to the centile limit of systolic BP found in different age in the population sample. There was a slight trend towards a higher BP in women with AP. Thus 17 women with AP were found in the upper and 12 in the lower 5 deciles of systolic BP. The overall presentation of women in the upper 5 deciles was not statistically significant. The result of the statistical analysis is the same if the women on antihypertensive treatment who were found in the third decile are taken to the upper 5 deciles.

Coronary ECG The preponderance of values above the median (Table V) was more marked in the women with coronary ECG than in those with AP and was statistically significant ($p < 0.01$). Eleven of 23 women with coronary ECG were on antihypertensive therapy and another two were on diuretics for other reasons.

Table V Women with AP (n = 29) and with coronary ECG (n = 23) placed in deciles of systolic BP as found in the population study (Fig 1) irrespective of whether on antihypertensive drugs or not (those on antihypertensive treatment also separately in brackets)

Decentile of systolic BP in the seated position									
1	2	3	4	5	6	7	8	9	10
Angina pectoris									
3	2	3	2	2	4	3	3	2	5
		(1)				(1)	(1)	(1)	(1)
<hr/>		12 (1)		17 (4)					
Coronary ECG									
1	1	1	2	0	3	1	3	3	8
			(1)		(1)		(3)	(1)	(5)
		5 (1)		18 (10)					

BP in women with MI

A history of arterial hypertension (elevated BP in connection with pregnancy only not included) was found in 22 of 47 women with MI who were survivors on arrival in hospital compared to 71 of 578 women (12 %) in the reference group. The difference was statistically significant ($p < 0.001$). Sixteen of 47 women (34 %) with MI were on antihypertensive drugs compared to 37 of 578 in the reference group (6 %) the difference being statistically significant ($p < 0.001$). Information was obtained concerning 7 women who died from an attack of MI but who were not hospitalized for suspected MI. Five of the 7 women (71 %) had a history of hypertension.

The systolic and diastolic BP in the women 3 and 12 months after the MI are shown in Figs 4 & 5. After the infarction there was no definite difference in BP between these women and those of the population sample. However 10 of 38 (26 %) were on antihypertensive drugs 3 months after the MI compared to 37 of 578 (6 %) in the reference group. Nineteen of 40 (48 %) were on antihypertensive drugs 1 year after the MI.

Family history of arterial hypertension

According to the format on genetic arterial hypertension was reported in one on both parents in 11 of 29 women with AP (38 %) and 9 of 23 women with coronary ECG (39 %). Eleven of 41 MI survivors (63 %) compared to 208 of 548 women (38 %) in the population sample. The MI group differed statistically significantly from the general population of women in this respect ($p < 0.001$).

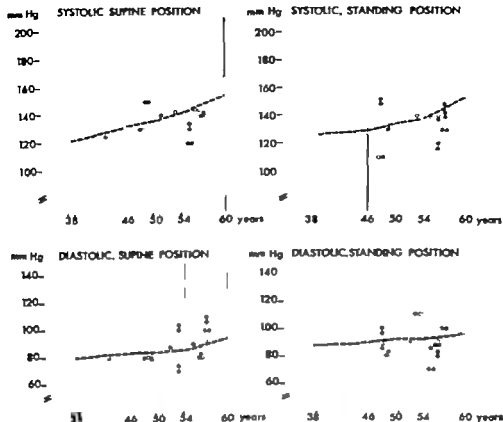


Fig 4 BP in the supine (n 38 data on 4 survivors missing) and standing (n 37 data on 5 survivors missing) position in women with MI 3 months after first infarction. The dotted lines combine the median value of the population sample of women in the age group 38 46 50 54 and 60. Filled symbols denote the on antihypertensive treatment.

while the women with AP and the women with a normal ECG did not. Arterial hypertension was reported in one or both parents of 4 of 6 women who died without being hospitalized for MI and in whom a family history had been obtained.

DISCUSSION

The BP values found in the present study were in agreement with the results of previous studies of women in Scandinavia^{34, 7} but somewhat lower than in Framingham U.S.A.³² and in Tecumseh U.S.A.³⁹ They were similar to those found in men in Göteborg.⁴²

The attendance rate was high in the present population study of women, being 90% of those drawn from the general population.⁵ This means that the data on the BP obtained from the population study are valid for the total population in the age group. This is further strengthened by the information obtained about the attendance rate. About the same proportion of women among the

Table V Women with AP (n = 29) and with coronary ECG (n = 23) placed in deciles of systolic BP as found in the population study (Fig 1) irrespective of whether on antihypertensive drugs or not (those on antihypertensive treatment also separately in brackets)

Decile of systolic BP in the seated position									
1	2	3	4	5	6	7	8	9	10
Angina pectoris									
3	2	3	2	2	4	3	3	2	5
		(1)				(1)	(1)	(1)	(1)
		12 (1)					17 (4)		
Coronary ECG									
1	1	1	2	0	3	1	3	3	8
			(1)		(1)		(3)	(1)	(5)
		5 (1)					18 (10)		

BP in women with MI

A history of arterial hypertension (elevated BP in connection with pregnancy only not included) was found in 22 of 47 women with MI who were survivors on arrival in hospital compared to 71 of 578 women (12%) in the reference group. This difference was statistically significant ($p < 0.001$). Sixteen of 47 women (34%) with MI were on antihypertensive drugs compared to 37 of 578 in the reference group (6%) the difference being statistically significant ($p < 0.001$). Information was obtained concerning 7 women who died from an attack of MI but who were not hospitalized for suspected MI. Five of these 7 women (71%) had a history of hypertension.

The systolic and diastolic BP in the women 3 and 12 months after the MI are shown in Fig 4.5. After the infarction there was no definite difference in BP between these women and those of the population sample. However 10 of 38 (26%) were on antihypertensive drugs 3 months after the MI compared to 37 of 578 (6%) in the reference group. Ten of 40 (25%) were on antihypertensive drugs 1 year after the MI.

Frequency of arterial hypertension

A total of 103 women with arterial hypertension was reported in one or both parts: 11 of 29 women with AP (38%) and 9 of 23 women with coronary ECG (39%) and 2 of 41 MI survivors (5%) compared to 208 of 548 women (38%) in the population sample. The MI group differed statistically significantly from the general population of women in this respect ($p < 0.001$).

clusions from BP d t rminations after th MI must be drawn with caution. The impression that th r is no difference in BP betw n women with MI and those in th gen al popul tion (Figs 4 5) may be = t fo women who att nd Post MI clinic but this does not necessarily mean that th ir BP wa th sam before th infarction. Women with MI w al o mor oft n on antihypertensive drugs 3 and 12 months aft r th nfarct on than women in the g neral population.

Data concerning p xistent hypert n on a p obably mor valid for om parison. Women with MI w significantly mo e oft n on antihypertensive treatment b fo th infarct on than women n the g neral popul tion and mo e oft n had a history of hype t n ion. Th p val c of hypert n ion in th present eri s of women wa imila to that ported in women with MI (survivo) and sudd n d ath in Helsinki Finland¹⁵⁴

Subj ct with MI had h t pain befo the infarction mo e often than women in th gen al population¹⁷. This might hav mad th m vi it a doctor and hav their BP ch ck d mo fr quently whi = may induc bias in th int pr tation of the results. How v it will probably not xplain th diff enc found

Information c nc rning family hi tory m to b mo unbi s d and a th familial facto i consid ed to be important fo hyperten ion²⁴. It wa tud d in th p nt mat ial A ording to th information volunt red, the women with MI had hyperten ve parent gnificantly mo oft n than th wo men in th r f ren group

Th present study does not how a d finit a soc tion betw n AP and hyper tension in women although th sult f om Framingham⁷² indi at thi

A larg majority of th women with o onary ECG in th p sent study w r on antihypert = v t tment o had high BP. Only few women with o onary ECG w found to have oth manif tation of IHD¹¹. It i pos sible that th o onary ECG a defin d nth p ent study i not pe ifi f r IHD but rath r d i tinguish be subj cts with hypert nsion¹. However a th seems to b a conn ction betw n arterial hypert nsion and ath n l osi it i also po ible that th o onary ECG p symptomatic manif tation of IHD. Th finding that a family hi tory of hyp rt n i s wa not m r common in the women with o onary ECG than in th gen al population of women i difficult to xplain

A p o p e tive study with a f llow up of a ro ct onally studied popula tion would be th id al way to obtain info mation about th imp rtan c of fa to s such a i d BP. Th sult of th T um h and Framingham p o spective studi are of int rest in th context. Thus Epstein⁵⁵ port d f om the T cum h study that th incid n of IHD in middl ag d w men up to th ag of 60 wa about th sam regardl of th valu of thei initial diast li BP whil ft th g of 60 tho with a BP in th uppe quintil had an inci den of IHD ght time that in th low quintil. In Framingham¹⁰² the in i

dence of IHD was higher in women with a BP > 160/95 and even in women with BP > 140/90 than in women with a BP < 140/90 (women aged 30-62 when they entered the study). This tendency was also found when the incidence of MI and AP were studied separately. However, the numbers of women with IHD were small in these prospective studies. The low frequency of MI in middle-aged women makes such studies difficult. If a number of women with MI large enough for valid statistical analyses is required.¹

SERUM CHOLESTEROL AND SERUM TRIGLYCERIDE LEVELS IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Calle Bengt son Per Björntorp and Elisabeth Tibblin

Abstract The serum cholesterol level of women who had suffered myocardial infarction was not higher than in the general population of women. No difference in serum cholesterol levels of women with angina pectoris and ECG changes suggestive of ischaemic heart disease. The serum triglyceride values were significantly higher in women who had had myocardial infarction and in women with ECG changes suggestive of ischaemic heart disease. There was no significant overrepresentation of high serum triglyceride values in women with angina pectoris.

Prospective and retrospective studies have demonstrated a statistical association between high serum cholesterol values and ischaemic heart disease (IHD) in men.^{71, 94, 167, 168, 199} Some investigators have also found elevated cholesterol values in women with IHD.^{5, 130} The literature on serum triglycerides is not as extensive but does indicate an association between high triglycerides and IHD in men.^{72, 71, 94, 184}

The level of cholesterol and triglycerides in women aged 38-60 years found in a population sample of women in Göteborg as described in detail elsewhere and related to previous studies.¹⁸⁰ The purpose of the present paper is to compare the cholesterol and triglyceride values of women with angina pectoris (AP), women with ECG changes suggestive of IHD (coronary ECG) and women who had had myocardial infarction (MI) with those of women in the population sample in order to find out whether there are differences between women with these manifestations of IHD and women in the general population.

MATERIAL

Reference group A population study of women in Göteborg, Sweden, was performed in 1968-1969.^{1, 15} Women in the age groups 38, 46, 50, 54 and 60 totaling 1462 women were examined. These women constitute the reference group.

Women with AP Twenty-nine women in the population sample reported symptoms of AP and had been ordered to Rest.⁵⁵

Women with coronary ECG ECG changes defined as coronary ECG were recorded in 23 women participating in the population study.

Women with MI All people in Göteborg born 1913 or later who have had an acute attack of MI have been registered in the list of January 1968 at the Post-MI Clinic in Göteborg.^{1, 50} Forty-nine women who were under observation in the hospital were registered during the years 1968-1970. Of these

4 women died during the period of hospitalization and one soon afterwards. Forty two survivors were admitted to clinical controls 3 and 12 months after the MI attack.⁵ Another 14 women died from a probable IHD attack outside hospital or while hospitalized for reasons other than suspected MI during the period. Ten of these women were classified as MI death.¹

METHODS

Blood sampling of the women participating in the population study was performed after overnight fasting as described previously.¹⁵ Serum cholesterol and triglyceride concentrations of these women were determined according to the methods described by Levine and Zak² and Lofblan¹¹⁵ respectively. The analytical steps after the extraction of lipids were automated. The cholesterol and triglyceride values of the women with MI were determined according to the same methods during the period of hospitalization except in 10 patients whose cholesterol values were determined according to Franey and Amador⁶ with minor modifications. The values obtained with the latter method closely agreed with those obtained with the previous method.

The cholesterol and triglyceride determinations at the 3 month and 12 month controls after the MI were carried out according to the methods described by Cramér and Jakobson and Carlson¹² respectively.

The correlations between values obtained with the methods used in the population study and at the clinical controls are shown in Fig 1.

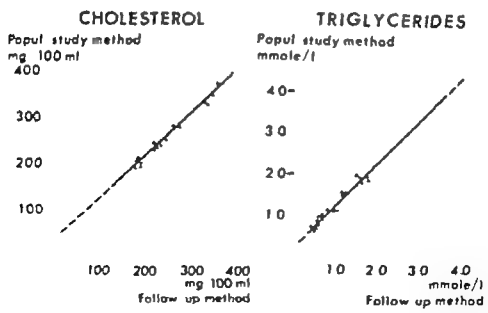


Fig 1. Correlation between cholesterol and triglyceride levels determined by the population study method and the follow-up method of the clinical controls.

Regression equations were for cholesterol $y = 0.918x + 32.5$ (mg/100 ml)

$r = 0.96$ and for triglycerides $y = 1.025x + 0.249$ (mmol/l) $r = 0.97$

The methodological error was calculated on duplicate analyses as $\sqrt{\frac{\sum d^2}{2N}}$ where d is the difference between duplicate and N the number of samples. The error of the method was then found to be 4.4 mg/100 ml for cholesterol and 0.05 mmol/l for triglycerides in the population study of 1968/1969 and 6.6 mg/100 ml and 0.04 mmol/l respectively in the follow up of 1978/1979.

Statistical method

Conventional statistical methods were used for calculation of mean values, standard deviation (S.D.) and correlation coefficient (r). The hypothesis of differences in frequency between groups was tested by means of the binomial distribution with a normal approximation.

Further details of material and methods are given in Chapter I.

RESULTS

Serum cholesterol and serum triglyceride levels in middle-aged women.

A detailed report on serum cholesterol and serum triglyceride levels in the present population sample is given elsewhere.¹⁰⁰ Means, S.D. and decile limits are shown in Table I and in Figure 2.3.

Table I Serum cholesterol and serum triglyceride levels
The Study of Women in Göteborg 1968/1969

Age (year)	n	Mean	S.D.
Cholesterol (mg/100 ml)			
38	371	241	34.8
46	429	260	53.6
50	398	276	41.9
54	178	284	41.7
60	81	283	35.0
Triglycerides (mmol/l)			
38	371	1.09	0.44
46	430	1.20	0.63
50	398	1.6	0.58
54	178	1.39	0.69
60	81	1.34	0.62

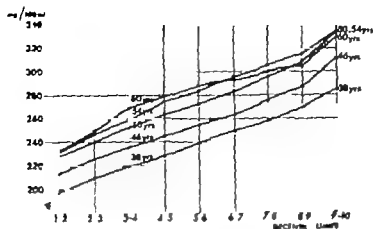


Fig 2 Dec ntile limit of serum cholesterol levels
The Study of Women in Göteborg
1968-1969

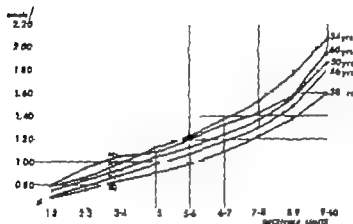


Fig 3 Dec ntile limits of serum triglyceride levels
The Study of Women in Göteborg
1968-1969

Angina pectoris. In Fig 4 the women with AP are placed in dec ntile limits of cholesterol and triglyceride values according to the value in the population study. There were no statistically significant overrepresentation of women with AP who had values in the upper 5 dec ntile limits (above the median) for triglycerides. Seventeen women had values above and 12 below the median. No difference was found for cholesterol (14 women who had AP were above and 15 were below the median). Only a few women had concomitant high cholesterol and triglyceride values. Nine women were found to have values above the median for both total cholesterol and triglycerides, while 7 women were found to have values below the median for both. Three of the women with AP were on lipid reducing drug (lofibrate) at the time of the examination. One of them had cholesterol and triglyceride values above the median and another who had previously had high cholesterol values was found to be below the median for both. The third woman had a cholesterol value below the median and a triglyceride value above the median. If the results in the women on lipid reducing drugs were discarded and their values were considered to be both below the median for cholesterol and

AP

"Coronary ECG"

CHOLESTEROL											Decen- tiles
1	2	3	4	5	6	7	8	9	10		
1	1	①		1		2				1	
2										2 1	
3									1	3	
4							1			4	
5			1	2	1				1	5	
6	1						1			6	
7			1		1	1	1			7	
8	1								1	8	
9			2	①			1		1	9	
10	1						1		1	①	
Decen- tiles											

CHOLESTEROL											Decen- tiles
1	2	3	4	5	6	7	8	9	10		
1										1	
2	1									2 1	
3										3	
4			1		1		1			4	
5			2	1						5	
6			1				1			6	
7									1	7	
8	2	1								8	
9	1					2			1	1	
10			1	1						1	
Decen- tiles											

Fig 4 Women with AP and coronary ECG classified according to deciles of serum cholesterol and serum triglyceride values as found in the population study (the first decile on lipid x during drug)

triglyceride is irrespective of the values found the statistical analysis would still have given the same results

Coronary ECG In Fig 4 the women with coronary ECG are placed in deciles of cholesterol and triglyceride values according to the values in the population study. Ten of them had values above and 13 below the median for cholesterol while 16 women had values above and 7 below the median of triglyceride. The overrepresentation of triglyceride values above the median was statistically significant ($p < 0.05$). None of the women with coronary ECG was on lipid reducing drug.

Serum cholesterol and serum triglyceride level in women with MI

Individual hospital and triglyceride values of women with MI at the time of the acute attack and at 3 and 12 months later are shown in Fig 5. Adjustments were made in the figures in order to compensate for the differences due to different methods of serum lipid estimation at the follow-up controls and in the population study. The number of subjects who had values above and below the medians of the population sample (for adjustment for the different methods of serum lipid determination) is summarized in Table II. There was a significant overrepresentation of women with triglyceride values above the median at the time of the acute attack and at the follow-up control afterwards. The cholesterol values of the women with MI were no higher than in the general population of women. Four of the women with MI were on a lipid reducing agent (lofibit) at the time of their MI. Two women after 3 months and 8 women after

CHOLESTEROL

TRIGLYCERIDES

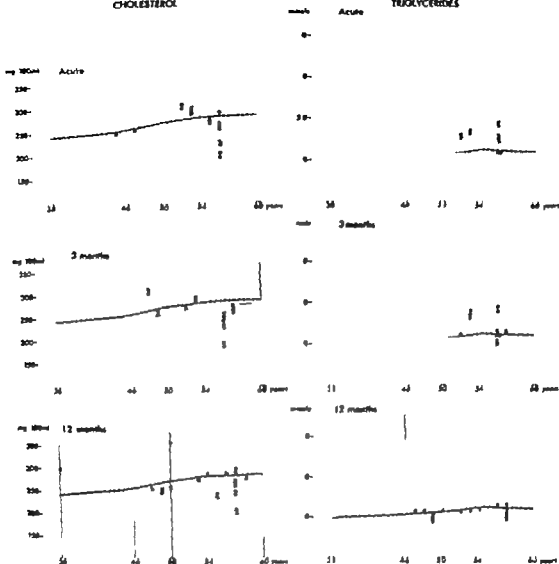


Fig 5 Individual serum cholesterol and serum triglyceride values in women hospitalized for MI at the time of the acute attack and 3 and 12 months later. Filled symbols denote women on lipid reducing drug. The line combines the median values of the female population aged 38, 46, 50, 54 and 60 as found in the population study before (—) and after (---) adjustment for the different method of serum lipid determination in the population study and at the 3 month and 12 month control.

12 months. If the values of the women who were on a lipid reducing drug were regarded as being above the median irrespective of the levels found, the results from the statistical analysis would still have been the same.

Table II Number of women hospitalized for MI who had values above and below the median for serum cholesterol and serum triglyceride levels in the population sample (number on lipid reducing drug is also given separately in brackets)

	Above median	Below median	Total	Significance of difference
Acute				
Cholesterol	15 (3)	23 (1)	38	NS
Triglycerides	29 (3)	8 (1)	37	$p < 0.001$
After 3 months ^a				
Cholesterol	14 (1)	23 (1)	37	NS
Triglycerides	34 (2)	2 (0)	36	$p < 0.001$
After 12 months ^a				
Cholesterol	19 (5)	20 (3)	39	NS
Triglycerides	36 (7)	3 (1)	39	$p < 0.001$

NS No statistical significance

^aAdjusted to compensate for the different methods of serum lipid determination at the 3 month and 12 month control and in the population study

A high cholesterol value was often not accompanied by a high triglyceride value in the same individual. For instance, of 20 women who had a triglyceride value in the upper 25% at the 3 month control, 8 were above and 12 below the median value of cholesterol at the same control.

Information was obtained about the serum cholesterol value of 6 women who died from MI outside the hospital. Two of them were known to have had values > 400 mg/100 ml.

DISCUSSION

Prospective studies of men have shown that a high cholesterol value is associated with an increased risk of MI as well as among others by Stamler⁶⁵, Carlson and Lindstedt³⁴ and Samberg⁶⁷. Prospective studies on triglyceride are few so far but give evidence that high triglyceride are associated with an increased risk of MI in men^{33, 34}. However, results from the study on men in Gotborg^{70, 6} that serum triglyceride value give no extra information for predicting the risk in men when serum cholesterol value are available.

Due to the low incidence of MI among young and middle aged women prospective studies are more difficult to perform in women, either a very large number or very long follow up period is necessary. In the prospective study performed in Framingham and Tübingen⁵ no distinction was made between the

various manifestations of IHD in women due to the low incidence rate. In the Framingham Study high cholesterol seemed to be a risk factor for IHD in women aged 30-49 but not in women aged 50-59 while triglyceride measured by means of an ultracentrifuge gave a better prediction of IHD than cholesterol levels in these older women.⁹¹ In the Tormo Study⁵⁵ no association was found between high cholesterol values and IHD in women.

Case control studies like the present one can probably give a somewhat false picture as the MI per se may in some way reduce the lipid values. Thus cholesterol and other lipids are known to fall after a MI and the values do not rise until several weeks later.^{67, 147} In a prospective study of men cholesterol and triglyceride values when measured 3 months after MI were similar to the preinfarction values for the same individuals¹⁰² which means that serum lipids measured 3 months after a MI are not much biased by the MI attack.

In the present study serum lipids were examined at the time of the attack and after 3 and 12 months when preinfarction values were thus expected. Although no low fat diet was prescribed initially the subjects themselves may have changed their dietary customs. Leiden¹¹⁰ noted a decrease in serum cholesterol of about 55 mg/100 ml in men who had had MI and who were prescribed dietary restrictions and a decrease of about 10 mg/100 ml in another group of men with MI to whom no instructions had been given. When abnormally high follow-up values were noted in the present study dietary advice and/or lipid reducing drugs were prescribed after the 3 month control. These possibilities of influence on the serum lipids must be kept in mind but are probably of minor importance. The different methods for serum lipid determination used in the present study are another possible source of error although efforts were made to compensate for these differences.

In the present study the women with MI or other manifestation of IHD were not found to have high cholesterol values than the women in the general population. Conclusions must be drawn with caution because of the facts discussed above. However, a serum lipid 3 months after the MI are probably not biased by the MI attack.¹⁰² It is unlikely that the cholesterol values of the women with MI were higher than in the general population even before the attacks. Triglyceride were significantly higher in the women with MI and in the women with coronary ECG and tended to be higher in the women with AP than in the general population.

In general the cholesterol and triglyceride values of the women with IHD were within the normal range or on the other showed an elevation. Only a few had coexistent high cholesterol and triglyceride values.

Cholesterol has in previous studies been found to be higher in women with MI⁵ and in women with IHD³² than in normal. It was also higher in hypertensive women with IHD than in hypertensive women free from IHD.⁵⁵ The

results from those studies that mostly disagree with the result from another case control study¹⁴⁷ in which no significant differences were found between women with MI below the age of 60 and their control from the prospective study in Framingham⁹³ and Tuncel⁵⁵ and from the present study. However, some of the risks were very small^{45,135} and none of the previous case control studies discussed used randomized subjects from the population as control. Mufson et al.¹³⁰ noted that the difference in serum cholesterol between women with IHD and controls was particularly prominent in the young age group (women aged 35-44) which agrees with the results from Framingham⁹³. There are too few women with MI below the age of 45 in the present study to allow separate conclusions concerning this age range.

The results of previous studies have shown that triglycerides tend to be higher or were significantly higher¹⁷ in women with MI than in controls and were higher in hypertensive women with IHD than in hypertensive women free from IHD.¹³⁹ The results generally agree with those from Framingham⁹³ and with the present result.

SMOKING HABITS IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Carl Bengtsson

Abstract Smoking was more common in younger than in older women in the general population as found from a population study of women aged 38-60. Almost half of the women aged 38 were smokers as compared to about 20 % of those aged 60. There was no overrepresentation of smokers in women who had angina pectoris or ECG changes suggestive of ischaemic heart disease. Women who had an acute attack of myocardial infarction were significantly more often smokers than women in the general population. In addition cigarette consumption was significantly higher among female smokers who had MI than among female smokers in the general population.

Smoking is considered to be associated with ischaemic heart disease (IHD)^{1,2}. Most previous information has been based upon studies of men with IHD. In the present chapter the smoking habits of women with manifestation of IHD are compared with those of women in the general population.

MATERIAL

Reference group From a population sample of women^{1,15} those of the age 50 and 54 (578 women) constituted the reference group. They had about the same mean age as the women with IHD¹. The smoking habits of the total sample (1462 women) are also described.

Women with angina pectoris Twenty-nine women participating in the population study reported symptoms of angina pectoris (AP) as defined according to Rose.¹⁵

Women with coronary ECG ECG changes defined as coronary ECG¹ were recorded in 23 women participating in the population study.

Women with MI All people in Göteborg born in 1913 or later who have had an acute attack of myocardial infarction (MI) have been registered since the 1st of January 1968.^{1,16} Forty-seven women who were survivors on arrival in hospital were registered during the years 1968-1970. Another 14 women died from a probable IHD attack outside hospital or while hospitalized for a cause other than suspected MI.¹⁷ 10 of whom were classified as deaths from MI.¹

METHODS

Interview concerning smoking habits

The women participating in the population study were interviewed about their smoking habit such as type of smoking (cigar ■ cigarette pipe) amount of daily consumption and for how long they had been smoking. They were also

asked whether they inhaled or not. Non smokers were asked whether they had smoked earlier in life and ex smokers were asked whether they had begun and when they had topped smoking. Women with MI were interviewed in the same way at the time of the acute attack. The daily consumption was recorded as 1, 14, 15, 24 or ≥ 25 cigarettes in the MI group. Subjects who smoked ≥ 1 cigarette per day were defined as smokers.

Statistical method

The hypothesis of difference in frequencies between groups was tested by means of the binomial distribution with a normal approximation.

Further details of material and methods are given in Chapter I.

RESULTS

Smoking habit in the population sample

Smoking was much more common in the young than in the old women (Table I). Almost half of the women aged 38 were smokers compared to about 20% of the women aged 60. The percentage of smokers was about 10% in the youngest and oldest women and about 6% in the middle three age strata. Only a small number of the ex smokers had stopped smoking during the previous year. More than 90% of the smokers were inhaling. Eleven women (about 1% of the women in the age group 38-54) never in the age group 60) smoked cigarettes and/or a pipe. Seven of them also smoked cigarettes. Table II shows the daily consumption of cigarettes among the cigarette smokers. More than half of the smokers smoked 5-14 cigarettes a day. The median daily cigarette consumption among the smokers was 10 cigarettes in the age group.

Table I. Smokers, ex smokers and non smokers (%)
The Study of Women in Göteborg 1968-1969

Category	Age (years)				
	38 n 372	46 n 431	50 n 398	54 n 180	60 n 81
Smokers inhaling	44.6	41.5	34.9	34.9	17.3
Smokers non inhaling	1.9	2.8	2.3	3.9	2.5
Ex smokers who topped smoking lately	1.1	0.9	0.3	0.6	3.7
Ex smokers who have not smoked during the last year	6.5	8	3.3	4.4	2.5
Ex smokers who have not smoked during the last 15 years	3.0	2.3	2.8	0.6	3.7
Non smokers (ex smokers not included)	42.7	49.7	56.5	55.6	70.4

Table II Cigarette smoking women (% of the total number of cigarette smokers) classified according to daily cigarette consumption and age
The Study of Women in Göteborg 1968-1969

Cigarettes/day	Age (years)				
	38	46	50	54	60
	n 173	n 189	n 147	n 69	n 16
1-4	10	18	15	20	19
5-9	0	29	28	9	19
10-14	36	28	39	33	37
15-19	18	15	9	11	19
20-24	13	8	■	6	6
≥ 25	1	2	1	0	■

Smoking habits in the women with AP and coronary ECG in the population sample

Angina pectoris Eight of 29 women with AP (28 %) were smokers and 6 (21 %) ex smokers. Fifteen women (52 %) had never been smokers. The percentage of women who had never been smokers is about the same as in the reference group. The percentage of smokers in the AP group is low and is lower than that of any group in the population sample except for the age group 60. Four of the 6 ex smokers had stopped smoking more than 6 years before the population study.

Coronary ECG Three of 23 women with coronary ECG (13 %) were smokers and 2 (9 %) ex smokers. Smoking was less common in the women with coronary ECG than in any of the age groups in the population sample.

Smoking habits in the women with MI

Information about smoking habits was obtained from 46 women with MI. Thirty-seven women (80 %) were smokers, 1 (2 %) was an ex smoker, and 8 (17 %) were non smokers (Table III). Smoking was much more common in the women who suffered MI than in the reference group. The women with MI who smoked were also significantly more often heavy smokers than the female smoker participating in the population study. None of the women with MI smoked cigars or a pipe. Confirmed information was obtained for only 3 of the women who died from MI outside hospital. One of these women was a smoker, one an ex smoker, and the third woman had never smoked.

Table III Smoking habits and cigar consumption in women hospitalized for MI compared to a reference group of women aged 50 and 54 participating in the population study

Category	Women with MI		Reference group		Significance of difference
	n	%	n	%	
Smokers	37/46 ^a	80	218/578	38	$p < 0.001$
Ex smoker	1/46 ^a	2	35/578	6	NS
Non smokers (ex smoker not included)	8/46 ^a	17	325/578	56	$p < 0.001$
Smokers and ex smoker	38/46 ^a	83	253/578	44	$p < 0.001$
≥ 14 cig/day ^b	1/35 ^c	60	177/216 ^d	82	$p < 0.001$
≥ 15 cig/day ^b	14/35 ^c	40	39/216 ^d	18	$p < 0.001$
Inhalers ^b	30/34 ^e	88	202/218	93	NS
Non inhalers ^b	4/34 ^e	12	16/218	7	NS

NS No statistical significance

^aInformation from one woman missing ^bAmong those smoking ^cInformation from 2 smokers missing ^dTwo women who smoked exclusively cigars or a pipe not included. ^eInformation from 3 smokers missing

DISCUSSION

A large number of communications have confirmed the association between tobacco smoking and IHD^{107-112, 175-196}. Autopsy findings have revealed a correlation between the degree of coronary atherosclerosis and the amount of cigarette smoking in men. The connection between smoking and IHD seems to be strongest for MI and more dubious for AP^{44, 61-144}. Nicotinic has been discussed as a possible causative agent by Kerschbaum et al¹⁰³ while Astrup et al¹⁰ have focused their interest on the possible atherogenic effect of carbon monoxide in smokers. However, it has been much debated whether the high incidence of MI in smokers is caused by smoking per se or if there exists some other common causative factor in smokers and subjects with MI, e.g. anxiety⁷⁹ or a special behavioural pattern^{1, 63, 196}. Results of twin studies, although based on limited data, do not confirm the theory of a direct connection^{64, 12, 117}.

The debate on the role of smoking as a risk factor for MI has been focused on men as they suffer MI more often than women. However, an increased mortality from IHD in smokers has been reported both in men and women⁸⁰. In the Framingham Study MI and fatal heart attack were more common in women who smoked than in those who did not, but the incidence of AP was similar⁸⁴. In a study in New York (the HIPP study) the incidence of MI was

significantly high in women who smoked than in those who did not but the incidence of AP was almost the same.¹⁶² The data from these studies agree with the observation from the present study in Göteborg that smokers were strikingly over represented among the women with MI but not in the women with AP.

Several of the women with AP were ex smokers. They had usually stopped smoking several years previous to the population study which means that most of them had not stopped smoking as a consequence of chest pain.

There was no over representation of smokers in the women with "coronary ECG" in the present study. On the contrary, smokers tended rather to be under represented in this group. This may possibly be another indication that the "coronary ECG" in women as defined is not mainly a manifestation of IHD.¹¹

Smoking of cigarettes was found to be about as common in the present study of women in Göteborg as in previous studies of women in Framingham U.S.A.⁴⁶ Bergen Norway⁴⁸ and Glostrup Denmark.⁴³ In Glostrup a further 18% of women aged 50 smoked cigars and/or a pipe compared to 1% of the women in Göteborg of the same age.

Smoking has been shown to be continuously on the increase in women²⁹ and is more common in the younger than in the older age group as noted previously^{22, 48} and in the present study. The difference in smoking habit between young and old women gives rise to an interesting question. Will MI be more common in women when the young women of today who are heavy smokers reach what may be called "maturational age"?

DIABETES MELLITUS CARBOHYDRATE TOLERANCE AND EARLY INSULIN RESPONSE TO AN INTRAVENOUS GLUCOSE INJECTION IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Calla Bengtsson Göran Blohmé and Johan Waldenström

Abstract The prevalence of manifest diabetes mellitus was significantly higher in women with myocardial infarction than in women in the general population. This was not the case for women with angina pectoris or ECG changes suggestive of ischaemic heart disease. There was no difference in fasting blood glucose or fasting serum insulin values between non-diabetic women who had angina pectoris or who had had myocardial infarction and non-diabetic women in the general population. Intravenous glucose injection did not reveal significant differences in glucose tolerance or mean values of early insulin response. However, there was an overrepresentation of women with a very low early insulin response in the women who had had myocardial infarction.

Manifest diabetes mellitus as well as isolated groups of impaired glucose tolerance have been found to be associated with ischaemic heart disease (IHD)^{14 144 148 193}. A decreased and delayed glucose stimulated early insulin response is considered to be a common feature in subjects with diabetes^{77 109}. The insulin response has usually been found to be either normal or increased in subjects with IHD^{78 120 2 33}. The purpose of this present chapter is to bring forth further data on this matter. Women with myocardial infarction (MI) and women in the general population in Göteborg compared with regard to the prevalence of manifest diabetes, tolerance to an intravenous (i.v.) glucose injection and early insulin response to such an injection. Some supplementary data are given concerning women with angina pectoris (AP) and women with ECG changes suggestive of ischaemic heart disease (conary ECG).

MATERIAL

Reference group A population study of women in Göteborg Sweden was performed in 1968-1969. Women in the age groups 38-46, 50-54 and 60 were examined in all 1462 women^{1, 5}. Except for a small subsample of women aged 50 were admitted to subsequent studies of the tolerance to i.v. glucose injection and the early insulin response^{20 21} (355 women participation rate 85.3%). The mean age of the women was at the time of the i.v. glucose tolerance test (IVGTT) 50.9 years. Two women with previously known manifest diabetes stated only with carbohydrate restriction and one woman with previously unknown fasting hyperglycaemia was studied, while two women inulin was excluded from the study. Seventeen (4.8%) of the women who were admitted to the IVGTT had continuously been on treatment during

th population before the test

When comparing the prevalence of manifest diabetes the women aged 50 and 54 in the population study totaling 578 women were chosen as a reference group as they had about the same mean age as the women with MI. A comparison of fasting venous blood glucose levels, glucose tolerance, fasting insulin levels and early insulin response was made between the women with MI and the 50 year old women participating in the population study. IVGTT had been carried out only in the 50 year old women in the population sample.

Women with AP and "coronary ECG" in the population sample. Twenty nine women in the population sample had AP as defined according to Rose.¹⁵ Twenty three women had ECG changes defined as coronary ECG.¹ The prevalence of manifest diabetes and fasting blood sugar values were studied on all subjects with AP and coronary ECG. IVGTT was performed only on those aged 50 in the population sample which means that 11 of 29 women with AP and 3 of 23 women with coronary ECG were examined by means of this test. The latter group of 3 women is considered too small to include in the presentation of results.

Women with MI. All people in Göteborg born in 1913 or later who have had an acute attack of MI have been registered since the 1st of January 1968. Forty seven women who were survivors on arrival in hospital were registered during the year 1968-1970.^{1,50} Another 14 women died from a probable IHD attack outside hospital or while hospitalized for reasons other than suspected MI.¹⁷ 10 of whom were classified as MI deaths, the others as coronary deaths.¹

Thirty six of the women with MI were admitted to studies of glucose tolerance and early insulin response on average 16.3 months (range 3-36 months) after their MI attack. They were then aged 45-58 (mean age 54.1 years). One of these women had manifest diabetes. She was treated with fenformin at the time of the MI attack but had been on dietary restrictions for two years when the IVGTT was performed. Five other women with manifest diabetes treated with insulin or oral hypoglycemic agents were not studied further. Seventeen (47%) of the women with MI who were admitted to the IVGTT had been on alaractics during the period before the IVGTT.

METHODS

Information as to the prevalence of manifest diabetes was obtained from interviews. The women were considered to have manifest diabetes if before the population study or the attack of MI they had been prescribed dietary restrictions and/or other treatment for diabetes. All of them had had glycosuria and elevated fasting blood glucose values. On women in the population sample and on women in the MI group with previously unknown diabetes had marked fasting hyperglycemia and glycosuria and were considered to have manifest

diabetes. The woman with MI gave a history of g at thir t for a long time befo the MI attack and ha sin the attack been tr at d with insulin

A fa ting v nou blood glucos valu of all participant in the population tudy was det rmin d by means of a ferric cyanid reduction m thod⁴² adapted for autoanalyzer by T chmi on N 26 Th methodological error ($\sqrt{\frac{\sum d^2}{2N}}$ d diff r nces betwe n duplicate N = number of samples) was 3.0 mg/100 ml

The wom n who w admitt d to the IVGTT were all ambulatory and came to th laboratory in the morning after 12 hours fasting Salur tl were with drawn for 1 ½ days and smoking was not allow d during the last 12 hou befor th test Glu os 0.5 g/kg body w ight in a 50 % solution wa inj cted intra v nously during 2 3 3 0 minut s Z ro time was et at th tart of the inje tion. Immediat ly before th injection and at 4 6 and 8 minut venous blood sample w re taken fo blood glucos and serum insulin det rminations Betw en 25 and 60 minut venou blood samples w tak n every 5 minutes fo glucos d t minations Blood glucos was analysed within 2 3 hours Serum fo th in ulin determination was tored at 20°C in small aliquot for later analys s

In the spe imens withdrawn befor and during the IVGTT glucos wa d ter mined in venou blood with glu os oxida using a commercially availabl reag nt (Glox[®] AB Kabi, Stockholm) and with glycín buff r d pe chloric acid as p ot in precipitating ag nt⁴³ Th methodological error wa 1.6 mg/100 ml The tol ran to i v glucos inj tion was expre d as a K valu (% d clin per minute) obtained from th slope of total blood glu o on a logarithmic s al from v nou blood glu os values betwe n 25 and 60 minut s originally d rived by Hamilton and St in⁷⁹ The best fit of th traigh t line wa det r mined by minimising the sum of square of the devi tion of th data point f om the lin Blood glucos valn s 10 mg/100 ml o l ab ve the fa ting l vel w re x lud d becau valu app oximating the fa ting l vel often deviat f om lin arity Serum in ulin wa determin d with a doubl antibody method orig nally de rived by Hale nd Randl⁷⁵ sing a omma cial radi immun assay kit (Th Radioch mu al C ntr Am ham U K) The tandard curv wa on tru t du ing human nsulin whi h wa d live d together with the kit. Th rum was ncubated during 6 18 hour t +4°C A omparative tudy show d that inc ng th ond incubation to 40 hour r sult d in about 20 % highe valu Nothing was gain d in pr l ion Th methodological error al cul ted from dupl at d t rminations was 1.4 mU/l at the 20 mU/l level and 3 mU/l at th 55 mU/l l vel A control containing 17 mU/l wa n lys d each day The day to day variat n ($\frac{SD}{\text{mean}} \times 100$) wa found to be 11 %

R l tive body w ight was expre s da % of d l w ight⁷

Details of the IVGTT a p nt d parat ly^{20 21}

Statistical methods

Conventional statistical methods were used for calculation of mean value standard deviation (S.D.) and correlation coefficient (r). Significance of differences between mean values was estimated by Student's *t* test. As the distribution curve of fasting serum insulin values and serum insulin values at 4, 6 and 8 minutes were skewed^{20, 21} but the log values normally distributed, the *t* test was applied to the means of log values for this variable. The distribution curve was also skewed for the values of serum insulin increase at 8 minutes while the log values were normally distributed. As some values were negative a non-parametric test (Mann-Whitney's U test)¹⁶ was applied for this variable. The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation. Further details of material and methods are given in Chapter I.

RESULTS

Prevalence of manifest diabetes mellitus

The population sample (Table I) shows the prevalence of manifest diabetes in the population sample. It was found that 0.8% of the total number of women studied had manifest diabetes. Among the women in the 50 and 54 years age group 6 women (1.0%) had manifest diabetes.

Information was obtained by interview or by studying inpatient and outpatient records from 119 women who refused to participate in the population study and from 93% of the responders. None of them had manifest diabetes.

Table I. Manifest diabetes prevalence and mode of treatment in women hospitalized because of MI and in a population sample of women

Age (years)	Subjects at risk n	Diabetic n	Treated n	Insulin n	Previously unknown n	Total n	%
Women with MI							
43-57	47	1	2	3	1	7	14.9
Women in the population sample							
38	372	0	0	1	0	1	0.3
46	431	0	0	0	0	0	0
50	398	2	0	2	1	5	1.3
54	180	0	1	0	0	1	0.6
60	81	3	2	0	0	5	6.2
Total	1462	5	3	3	1	12	0.8

Women with AP No of the women with AP had manifest diabetes
Women with coronary ECG One of 23 women with coronary
ECG had manifest diabetes

Women with MI Seven of 47 women hospitalized for MI (15%) had
manifest diabetes This may be compared to six of 578 of the women aged 50
and 54 who were participating in the population study (1.0%) The difference
is statistically significant ($p < 0.001$) The diabetic women with MI were
on insulin at the time of their MI and two were on oral hypoglycemic agent
One woman on diet and one previously untreated woman was given insulin at
the time of the MI attack Except for one insulin-treated woman aged 37 the
age range of the diabetic women with MI (45-54 years) was similar to the rest
of the MI group (43-57 years Table I)

Two of 10 MI deaths outside the hospital or while hospitalized for reasons other
than MI and one out of another 4 deaths classified as coronary deaths had
manifest diabetes Thus in total 10 of 61 women (16%) who were hospitalized
because of MI or died from a probable IHD attack outside hospital or while
hospitalized for reasons other than MI had diabetes

Four women hospitalized for MI died during the period of hospitalization
Two of them had diabetes If they are included the primary mortality was
found to be 50% in 10 diabetic women and 25% in 51 non-diabetic women among
those 61 women who were hospitalized for MI or died from a probable IHD
attack without being hospitalized for MI The difference in primary mortality
between diabetic and non-diabetic women is not statistically significant

Fasting blood glucose

Mean fasting venous blood glucose was similar in the groups of women with
AP coronary ECG and in the reference group of women aged 50 when measured at the
population study (78.4, 75.2 and 75 mg/100 ml S.D. 14.4,
16.2 and 16.7 mg/100 ml respectively) It was also similar in the MI group
and in the reference group (Table II) The specimens withdrawn when performing
the IVGTT Women on salutarics did not differ from those who were not on
salutarics

Glucose tolerance

Mean K values of the women who had had MI and of the women in the reference
group did not differ significantly (Table II) In Table III the women are sub-
divided according to their K values Table III also includes 50-year-old women
with AP Low K value (< 1.00) tended to be over-represented in the women
with MI and in the women with AP compared to the reference group but the
differences were not significant There were no differences between women
who took and those who did not take salutarics either in the IHD groups or in

Table II Fasting v nous blood glucose values K value s values for fasting serum insulin serum insulin 4 6 and 8 minutes after i v glucos injection s rum insulin incre se and body w ight in 36 women who had had MI and in a ref erence group of 355 50 year old women

	Wom n with pr vious MI			R ference group		
	Mean	S D	Range	Mean	S D	Range
Fasting blood glucose ^a (mg/100 ml)	71 0	12 9	52 109	70 2	8 0	55 105
K value (%/min)	1 64	0 84	0 55 4 74	1 87	1 10	0 49 9 86
Fasting serum insulin (mU/l)	15 1	9 1	4 42	14 0	5 3	3 47
Serum insulin 4 min (mU/l)	83 3	53 7	12 410	86 2	47 0	12 390
Serum insulin 6 min (mU/l)	69 6	45 0	16 250	72 1	45 7	12 495
Serum insulin 8 min (mU/l)	57 1	37 8	11 110	60 6	37 4	14 380
Serum insulin incre se at 8 min (mU/l)	41 9	36 6	3 176	46 6	35 6	11 333
Body weight (kg)	66 1	9 8	50 8 90 8	67 2	11 4	44 7 112 4
Relative weight (%)	108 2	16 1	82 144	108 9	16 9	76 174
Log K value	0 163	0 215		0 218	0 205	
Log fasting serum insulin	1 116	0 235		1 116	0 159	

^aOne woman in the MI group and 3 women in the r ference group w th manif st diabetes are excluded

the reference group

Fasting e um nsulin

The fasting serum insulin value s of the women who had had MI did not diff r significantly from tho n th reference group (Table II) no did tho of the 50 year old wom n with AP (mean 16 3 mU/l S D 11 mU/l if one woman with a v ry high value 47 mU/l s ex luded) The corr lation coeffi nt (r) between fasting insulin and lat v body w ight was found to be 0 31 in the r f erence group and 0 33 in the MI group

Table III Distribution of K values in 35^a women with previous MI aged 45-58 in 11-50 year old women with AP and in a reference group of 35^b 50 year old women

K value	Women with MI		Women with AP		Reference group	
	n	%	n	%	n	%
< 0.90	4	11	0	0	21	6
0.90-0.99	3	9	2	18	18	5
1.00-1.09	2	6	1	9	22	6
≥ 1.10	26	74	8	73	291	83

^aOne woman with manifest diabetes excluded. ^bThree women with manifest diabetes excluded.

The fasting insulin values were significantly higher in the women on saluretics than in those who were not on saluretic in the MI group (mean 19.2 and 11.5 mU/l, respectively $p < 0.01$). They tended to be higher in those on saluretics than in those who were not on saluretic in the reference group (17.4 and 13.8 mU/l respectively $p < 0.10$). The mean relative body weight was higher in the reference group among women on saluretics (118 % as compared to 108 % among those who were not on saluretic $p < 0.05$). In the MI group there was no significant difference (108 % and 109 % respectively). However, 4 women on saluretic with a relative weight ≥ 130 % and high fasting insulin values of 25-35 mU/l mainly contributed to the higher mean value of fasting serum insulin in the women on saluretics in the MI group.

Serum insulin response

Mean serum insulin levels measured 4, 6 and 8 minutes after the beginning of the i.v. glucose injection in the women with MI and in the reference group are shown in Table II. No significant differences in mean values were found. No did the value for serum insulin increase at 8 minutes differ significantly between the groups. However, 9 of 35 non-diabetic women (26 %) in the MI group had an insulin increase at 8 minutes within the lowest decile of the reference group (≤ 16 mU/l). This overrepresentation of women with a very low early insulin response in the MI group was statistically significant ($p < 0.01$ two-tailed test).

The correlation coefficient (r) between K value and insulin increase at 8 minutes was found to be 0.40 in the reference group and 0.42 in the MI group. Non-diabetic women who had both a low K value (< 1.00) and a very low insulin increase after 8 minutes (≤ 16 mU/l) were overrepresented in the MI group (14 % as compared to 4 % in the reference group $p < 0.01$ two-tailed test) while women with a low insulin increase after 8 minutes but a K value ≥ 1.00

were more similarly distributed in the non diabetic MI group and in the reference group (11 % and 6 % respectively). No differences were found in body weight or relative body weight between the MI group and the reference group (Table II) and there was no difference in the glucose given as this was proportional to the body weight. Women on saluretics did not differ from those who were not on saluretics.

The value for serum insulin increase at 8 minutes in the 50 year old women with AP did not differ significantly from those in the reference group. They were very high in 2 women (333 and 169 mU/l) one of whom also had a very high fasting serum insulin value. The others had values similar to those found in the reference group (mean 41.4 mU/l \pm SD 15.2 mU/l).

DISCUSSION

The prevalence of manifest diabetes in women aged 50-54 was 1.0 % in the present population sample which is in agreement with previous reports^{24, 52, 69}. Due to the high participation rate and the information concerning diabetes being available for such a large number of non participants the information on the prevalence in the population studied is considered to be valid.

There are too few women with angina pectoris in the present population study to allow valid conclusion about this group. The fact that none of the women with angina pectoris had manifest diabetes does not indicate an association between angina pectoris and diabetes in women. This agrees with the observation from the Framingham Study⁷⁵ that AP was only slightly more common in women with diabetes than in non diabetic women.

The prevalence of manifest diabetes in the women with MI was significantly higher than in women in the general population. This is in agreement with previous reports^{17, 26, 62, 63, 124, 75}. Garcia et al.⁶⁴ reported from Framingham that IHD is especially frequent in diabetic women on insulin but a high rate of IHD in subjects who have mild diabetes of late onset has also been reported.¹⁷⁵ Death from IHD was found to be more common in women with diabetes than in non diabetic women in the Framingham Study⁷⁶. A similar tendency was seen in the present study. Domenege et al.³ stated from autopsy findings that diabetes is the most important single atherogenic factor.

Diabetes is a predisposing factor to atherogenesis and has been considered to be a common feature in subjects with IHD as reviewed by Wahlberg.⁷⁷

An abnormal tolerance to glucose injection was not significantly more common in non diabetic women who had had MI than in the reference group in the present study. This agrees with the results of most previous studies which have been carried out exclusively or predominantly on men with IHD.^{70, 119, 122, 140}

In a study carried out by Wahlberg⁷³ abnormal or borderline K values were significantly more common in women who had had MI than in a female control

group. The women who had had a MI had a mean age of 66 years and were on average 11 years older than their control. Low K values were more common in older than in younger subjects in the MI group (men and women) but not in the control group (men and women). The women who had had MI in that trial were on average 12 years older than in the present study. It is thus possible that an impaired tolerance to intravenous glucose injection is a more common feature in older subjects who have had MI and the discrepant results in different studies might be explained in this way. The time between the MI attack and the IVGTT may be another factor of importance. Most subjects in the study presented by Wahlberg¹³⁹ were tested 3-6 weeks after their MI. Re-testing later on did not reveal any significant change in glucose tolerance. In another study¹⁴⁰ impaired tolerance to intravenous glucose injection was common immediately after an acute attack of MI (1-23 days) but normalization was usually not achieved after 6-12 months.

Fasting insulin has been found to be similar in subjects with MI as in controls^{141, 142, 143} or higher¹⁴⁴. In the present study of women ranges and mean of fasting serum insulin values were similar in the MI and in the reference group. In agreement with previous observations¹⁴⁵ obesity was correlated to high fasting insulin values in the present study. The higher fasting insulin values in women who before the IVGTT had been on saluretic were probably explained by a higher degree of obesity in the women.

Women who took and women who did not take saluretics were studied separately in a previous study¹⁴⁶ have suggested a possible association between impaired glucose tolerance and saluretic^{147, 148} and almost half of the non-diabetic women with a previous MI took saluretics compared to only about 15% of the non-diabetic women in the reference group. With the exception of fasting insulin values no difference was found between the who took and those who did not take saluretics for the variables studied with the IVGTT.

Abnormally high insulin response after oral glucose loading has been reported in series of predominantly male subjects with IHD^{149, 150, 151, 152}. After intravenous glucose injection to subjects with MI the early insulin response has been found to be normal¹⁵³. In the present study the range of early insulin values was wide both in the MI group and in the reference group. The mean values at 4, 6 and 8 minutes did not differ significantly between the groups.

The turnover of insulin in peripheral blood is rapid^{154, 155}. The insulin in circulation at 8 minutes when the calculation of the early insulin response in the present study does not take into account the insulin response. The insulin response can be calculated approximately from the insulin values during the initial phase of the test^{156, 157}. However, this is a high correlation ($r = 0.99$) between the insulin increments at 8 minutes and the early insulin response during the first 8 minutes calculated in this way.¹⁵⁸

The values for early insulin increase at 8 minutes did not differ significantly between women with MI and women in the population in the present study. However, women with no or very low early insulin increase were over represented in the MI group. An increased and delayed early insulin response is considered to characterise not only manifest diabetes and lesser degrees of impaired glucose tolerance but also the so called pre-diabetic state.³⁷ The findings might indicate an association between the pre-diabetic state and IHD.

NUMBER OF PREGNANCIES USE OF ORAL CONTRACEPTIVES AND MENOPAUSAL AGE IN WOMEN WITH ISCHAEMIC HEART DISEASE COMPARED TO A POPULATION SAMPLE OF WOMEN

Call Bengtson Göran Rybo and Hans Westberg

Abstract The number of childbirth had been significantly greater in women with angina pectoris or ECG changes suggestive of ischaemic heart disease than in women of the general population. A similar tendency was found for women with myocardial infarction. Spontaneous and/or legal abortions had been significantly more common in women with myocardial infarction. None of the women with myocardial infarction, angina pectoris or ECG changes suggestive of ischaemic heart disease had ever used oral contraceptives. Eight women who had a myocardial infarction before the age of 48 were still menstruating when they had their infarction. At respective menopausal age it was found that significantly more women who had suffered myocardial infarction were postmenopausal at the age of 45 and 50 than women in the general population. Menopause at the age of 50 or earlier had also taken place significantly more often in women with angina pectoris and in women with ECG changes suggestive of ischaemic heart disease. Postmenopausal 50-year-old women had higher serum cholesterol and serum triglyceride values than 50-year-old women still menstruating but blood pressure was lower than in the latter group of women. The differences in serum lipids and blood pressure between premenopausal and postmenopausal women were statistically significant.

The fact that myocardial infarction (MI) is rarer and occurs at a later age in women than in men has usually been explained by the hypothesis that women are in some way protected from MI by ovarian hormones¹⁷⁰. It has also been stated that women with a late menopause are more prone to MI than women in whom the menopause occurs late in life. The role of ovarian hormones in the development of MI has also been discussed in connection with pregnancy and the use of oral contraceptives.

The purpose of the present report is to compare women with manifestations of ischaemic heart disease (IHD) to the general population of women in a defined geographical area with regard to number of pregnancies, use of oral contraceptives and age at the menopause.

MATERIAL

Reference group A population study of women in Göteborg, Sweden, was performed in 1968-1969^{1,15}. Women in the age groups 38, 46, 50, 54 and 60 were examined. 1462 women in all. The age groups 50 and 54 (578 women) constituted the reference group in this present chapter. They had about the same mean age as the women with IHD¹.

Women with angina pectoris Twenty-nine women participating in the population study were found to have angina pectoris (AP) as defined according to Ros¹⁵⁵.

on the other women. In view of their age they probably did not use oral contraceptives (one of them was 42 years old and all others ≥ 48 years old).

Premenopausal and postmenopausal women

Figure 1 presents the women who had MI by age and menopausal status. All women who were 47 years of age or younger were still menstruating at the time they had their MI. However, women with MI usually had menopause at a younger age than women in the reference group (Table III). A retrospective comparison revealed that a significantly larger number of women were postmenopausal at the ages of 45 and 50 in the MI group than among women in the general population. Similarly, significantly more women with AP and women with coronary ECG were postmenopausal at the age of 50. Information was obtained for 4 women who died from MI outside the hospital. Their menopause occurred at 43, 47, 50 and 51 years respectively.

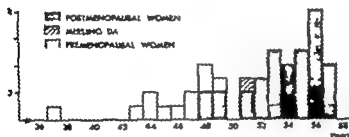


Fig 1 Women with MI survivors on arrival in hospital, classified according to whether menstruating or not.

Table III Number of hospitalized women with MI and number of women with AP and coronary ECG who had reached the menopause at the age of 40-45 and 50 respectively compared to a reference group of women aged 50 and 54 participating in the population study (% in brackets)

	Women with MI	Women with AP	Women with coronary ECG	Reference group
Menopause at the age of 40 or earlier	4/47 (9)	2/28 (7)	2/22 (9)	23/578 (4)
Menopause at the age of 45 or earlier	10/46* (22)	5/28 (18)	5/22 (23)	67/578 (12)
Menopause at the age of 50 or earlier	28/37** (76)	14/22* (64)	12/17* (71)	280/578 (48)

* $p < 0.05$ *** $p < 0.001$ significantly different from women in the general population.

Blood pressure and serum lipids in premenopausal and postmenopausal women

The 50 year old women in the population sample comprised about the same number of premenopausal and postmenopausal women. In Table IV a comparison is made between these two groups with regard to blood pressure and serum lipids which are usually considered as main risk factors for IHD. It is seen that the systolic blood pressure was lower in the postmenopausal women while serum cholesterol and serum triglycerides were raised in the women. The differences were statistically significant.

Table IV Mean, standard deviation (S.D.) and standard error of the mean (S.E.) of arterial blood pressure, serum cholesterol and serum triglyceride in premenopausal and postmenopausal 50 year old women

	Premenopausal women (n = 185)			Postmenopausal women (n = 148)			Significance of difference
	Mean	S.D.	S.E.	Mean	S.D.	S.E.	
Systolic blood pressure (mm Hg)	140	22.4	1.64	135	21.4	1.76	$p < 0.05$
Diastolic blood pressure (mm Hg)	88	10.5	0.77	88	10.9	0.90	NS
Serum cholesterol (mg/100 ml)	266	36.4	2.88	286	46.6	3.83	$p < 0.001$
Serum triglyceride (mmol/l)	1.16	0.52	0.04	1.34	0.67	0.05	$p < 0.01$

NS = Not statistically significant

DISCUSSION

In the present study the number of pregnancies tended to be greater in women with various manifestations of IHD than in women in the general population. Some of the differences were statistically significant. In disagreement with the results of a previous autopsy study failed to reveal an association between number of pregnancies and degree of coronary atherosclerosis.¹⁴ However, the results of the present study agree with those of three other clinical studies.^{17, 20, 209} The reason for a possible association between number of pregnancies and IHD is unknown. Influence of a larger number of pregnancies on cardiovascular body function may be one possible explanation. Epidemiologic studies by a greater number of children, both in a disproportionately larger number of spontaneous and/or legal abortions in women with MI has also to be considered.

According to results from previous studies²⁴ oral contraceptive may

be associated with an increased incidence of MI in women. In the present study no woman was on oral contraceptives in any of the IHD groups. Although these drugs were in common use in Göteborg. The present series is too small to exclude an association between oral contraceptives and MI but in conformity with two previous reports from Scandinavia^{25, 60} the result of the present series indicate that oral contraceptives are not a very important factor for development of MI in women.

Oophorectomy and spontaneous early menopause have been found to be associated with an increased incidence of IHD.^{29, 115, 120} An insignificantly excessive number of deaths from IHD was also found in a large study of women who were radiated because of menorrhagia³. Oliver¹³⁰ found that 20 out of 100 women with IHD who were aged 23-44 were postmenopausal before the development of IHD. Contrary to these results no association between oophorectomy or premature menopause and an increased risk of IHD has been found in some other studies.^{19, 124, 153} Wink and Hager¹⁰⁷ reported a study of 273 women with MI in which 9 women were 50 years of age or younger. These 9 women were all still menstruating when they had their MI.

The present study supports the view that there is an association between premature menopause and IHD. A significant number of women reached the menopause earlier than the women in the general population. However, all the women with MI aged 47 or less were still menstruating when they had their infarct, which means that menopause or oophorectomy is definitely not an obligatory factor for the development of MI at an early age.

As the postmenopausal woman seems to be associated with an increased risk of development of IHD, supplementation with estrogens might be expected to prevent IHD. The incidence of IHD has been reported to be reduced in women who were treated with estrogens for postmenopausal complaints¹²⁰ but there seems to be no other study which has shown that estrogen supplementation reduces the incidence of MI in women. A controlled prospective study is necessary in order to evaluate this treatment in women.

Paradoxically there are more data on the effect of estrogen treatment in men. Studies on the effect of estrogen treatment and its role in the prevention of IHD in men have given contradictory results. Favourable¹²¹ unfavourable⁷⁷ as well as inconclusive results¹ have been reported.

A hypothesis has been advanced that estrogens might influence coagulation and/or fibrinolytic mechanisms in raising the risk of thrombo-embolic disease but protecting from atherosclerosis and late atherosclerotic complications.³⁴ Such a hypothesis might explain a possible increased risk of thromboembolism including MI as a result of taking oral contraceptives as the estrogen component of them has been considered to be the most important in this respect.² The controversial result of estrogen treatment might also

be explained in this way. The hypothesis is agree with the observation by Stamler et al.¹⁷⁰ on estrogen-treated men that the mortality during the first period after starting estrogen treatment was unexpectedly high while the long term survival increased. The low incidence of MI in young and middle aged women compared to that of men might also be explained in this way.

Possible mechanisms of estrogen on the coagulation system and fibrinolysis have been discussed by Waxler et al.¹⁷⁴ and by Aitdt⁸ amongst others. It has also been postulated that estrogens interfere with adrenalin metabolism or its release from the sympathetic nerve.⁷ Estrogens are also known to reduce the serum cholesterol level^{171, 172} which is raised in postmenopausal women as shown in the present study and also previously.⁷⁷ However a protective effect via cholesterol is small especially as an increased level of serum cholesterol does not seem to be associated with an increased risk of IHD in women.⁷⁷ Nor is it probable that the significantly but moderately increased triglyceride level in the postmenopausal women is a factor of importance and the low blood pressure in the postmenopausal women should contribute to decrease rather than increase the risk of IHD in postmenopausal women.

hospitalization nor about those who died from MI outside hospital and only few comprehensive data were obtained on social factors in these women

METHODS

Collection of social data Information concerning marital status and place of birth of the women participating in the population study was obtained from the Revenue Office Register and by interview from the women with MI. Information about education, number of children, dwelling standard and socioeconomic group was obtained by questionnaire. The subjects were classified into five social groups according to Carlson¹⁰. Group I according to this classification included the present day social group I (large scale employees and officials of high or intermediate rank), groups 2 and 3 as social group II (small scale employees, officials of lower rank, foremen) and groups 4 and 5 as social group III (skilled and unskilled workers). Subjects occupied with domestic work but no work outside the home and subjects on a pension were classified into separate group. The classification of the women in the MI group and of the participants in the population study was made by the same person. Information concerning income during the year preceding the population study of the MI attack was obtained by interview. The total income was recorded. Pension was regarded as income. Status in congruity was defined as either high education of the woman (continued after elementary school) and low income of her husband (within the lower 75th percentile as found in the population study) or a low education of the woman (elementary school only) and high income of her husband (within the upper 3rd percentile as found in the population study).

Quantification of psychosocial stress factors A quantification of psychosocial stress factors during the year preceding the population study of the MI attack was obtained in an interview. In a standardized way the subjects were asked about ten groups of social events with negative significance: 1) severe illness of husband, 2) nervous symptoms in husband necessitating contact with psychiatrist or regular drug consumption, 3) husband a chronic alcoholic, 4) economic problems at husband's work, 5) legal separation, divorce or husband's death, 6) severe illness or very bad behaviour disturbance in child, 7) last birthday of husband, 8) severe illness or death of parents, 9) severe personal misfortune, 10) moved to new dwelling. A score of zero or one was given for every stressor group. Thus an individual could obtain a maximum stressor score of ten. It should be noted that some stressors have had an acute onset during the study period while others have exerted their influence during years. Further details about the stressor scale are presented elsewhere¹¹.

Assessment of subjective stress experience Information about subjective feeling of stress or strain was obtained in another interview independently of the stressor quantification described above and by another physician. The women were asked whether they had had a feeling of stress for a month or longer including tension, fear, anxiety or sleep disturbances in connection with conflicts in the family at work etc. Severe stress was defined as a continuous feeling of stress during the year preceding the population study or the MI.

Measurement of personality traits Information concerning personality traits was obtained from standard questionnaires. The participants in the population study and the women with MI answered Cosserac Mark's Personality Schedule (CMPS) which is a personality test similar to Edwards Personal Preference Schedule (EPPS)⁴⁶. Both CMPS and EPPS are based upon Murray's theory of personality.⁴⁷

CMPS consists of 165 questions covering 11 personality traits: achievement, affiliation, aggression, autonomy, definition of status, dominance, exhibition, guilt feelings, nurturance, order and succorance. After factor analysis the scale have been combined into five weighted indices: neuroticism, self assertiveness, rational dominance, aggressive nonconformance, passive dependency and sociability. For example, neurotic self assertiveness was calculated from the scores of the following primary traits: achievement (x6), guilt feelings (x5), definition of status (x5) and aggression (x4). Two variables in the CMPS: achievement and aggression were considered to measure central aspects of 'the coronary behavioural pattern Type A' described by Friedman and Rosnman.⁴⁸ The behavioural pattern Type A is a behavioural syndrome or a style of living characterised by extremes of competitiveness, job involvement, striving for achievement, aggression, haste, impatience, restlessness and tension.

The participant who had had MI were examined 6-36 months after the MI attack (mean 18 months). The participants of the population study including those with AP and coronary ECG were examined at the time of the study.

Statistical methods

The hypothesis of difference in frequency between groups was tested by means of the binomial distribution with normal approximation when studying the effect of the subjective experience of stress. In the study of personality traits Mann-Whitney's U test with correction for ties was utilized.⁴⁹ The statistics were studied with a generalisation of the test for trend in contingency tables⁵⁰ allowing within group matching of married and single women. Significance of difference between group means was estimated with Student's t test.

Further details of material and methods are given in Chapter I.

RESULTS

Social factors

Social factor in the women with IHD did not differ essentially from those in the reference group (Table I). However some tendencies may be noted. Thus there were comparatively few unmarried women in the various IHD groups. Significantly more women had 4 or more children in the MI group and in the group of women with coronary ECG. The socioeconomic status tended to be lower in the women with IHD than in the reference group, both the socioeconomic status of the women themselves and that of their husbands (Table II).

Table I. Marital status, number of children alive, place of birth and education in women hospitalized for MI, in women with AP and coronary ECG, and in a reference group of women aged 50 and 54 (%)

	Women with MI n = 47	Women with AP n = 29	Women with coronary ECG n = 23	Reference group n = 578
<u>Marital status</u>				
Unmarried	2	0	4	8
Married	77	79	91	76
Divorced	11	10	4	10
Widowed	11	10	0	6
<u>Number of children alive^a</u>				
0	20	10	22	21
1-3	61	76	61	72
≥ 4	18**	14	17*	7
<u>Place of birth^b</u>				
County of Göteborg	53	48	48	52
Remaining part of Sweden	38	45	48	43
Remaining part of Scandinavia	7	3	4	4
Remaining part of the world	2	3	0	1
<u>Education</u>				
Elementary school	76	86	87	71
Interrupted middle school	7	7	13	8
Middle school	10	3	0	17
Interrupted high school	2	3	0	1
High school	5	0	0	2

* $p < 0.05$ ** $p < 0.01$ significantly different from the general population of women

^aData on 3 women with MI missing ^bData on 2 women with MI missing ^cData on 5 women with MI missing

Table II Socio-economic group of women hospitalized for MI of women with AP and coronary ECG and socio-economic group of their husbands compared to a reference group of women aged 50 and 54 (%)

Socio-economic group	Women with MI n = 44 ^a	Women with AP n = 28 ^b	Women with coronary ECG n = 23	Reference group n = 578
Socio-economic group (according to the profession of the women)				
I	0	0	0	3
II	20	14	13	30
III	32	34	35	30
Domestic work only	36	34	35	34
Pension	11*	17**	17**	3
Socio-economic group (according to the profession of the husbands)				
I	5	0	0	14
II	38	48	20	41
III	46	52	75	44
Domestic work only	0	0	0	0
Pension	10***	0	5	1

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ significantly different from the general population of women (two-tailed test)

^aData on 3 women missing ^bData on one woman missing

The number of women working in the home only was about the same in all the groups while the number of women receiving pensions was significantly larger in the groups of women with IHD than in the reference group. Consequently the number of women working in the home was larger in the reference group than in the women with IHD. The husbands of the women in the MI group were more often receiving a pension than the husbands of the women in the reference group.

The income was similar in the various groups of women with IHD (Table III). About 70% in the reference group and about 80% of the MI group were receiving an income and/or a pension. The dwelling standard measured as the number of rooms was about the same in all the groups. No significant difference in situ incongruity between husband and wife was defined in the present study was not detected (1%, 37% and 20% among the married women in the groups of women with MI, AP and coronary ECG respectively, 25% among the married women in the reference group).

Table III Income + pension of women hospitalized for MI of women with AP and coronary ECG also the reference group of women aged 50 and 54 as well as those of their husbands if married

	Women with MI	Women with AP	Women with coronary ECG	Reference group
Income of the women (Sw. crowns)				
Median value	12000	10000	6000	14000
Income or pension (n)	33/4 ^a	18/28 ^b	14/23	388/555
% with income or pension	79	64	61	70
Income of the husbands (Sw. crowns)				
Median value	30000	28000	25000	30000
Women who had a husband with income or pension (n)	34/41 ^d	21/28 ^b	20/23	434/555 ^c
% with a husband with income or pension	83	75	87	78

^aData on 5 women missing ^bData on one woman missing ^cData on 23 women missing ^dData on 6 women missing

Information was obtained on marital status in 8 women who died from MI without being hospitalized for suspected MI. Five of them were married and 3 unmarried. Valid information was not obtained about the other social factors studied in those who died outside hospital or when hospitalized for reason other than suspected MI.

Psychosocial stressors

As unmarried women are not subjected to all the stressors studied and as the unmarried women to some extent are under represented in the hospitalized group the number of stressors in married and unmarried women are presented separately. As will be seen from Table IV the number of stressors tended to be large in both married and unmarried women with MI than in the reference group of women. Statistical analysis when married and unmarried women were matched pair wise revealed a significant difference between women with MI and women in the general population ($p < 0.05$). Except for stressor nr 7 (last child left home) every stressor was over represented in the MI group. The number of women who reported at least one stressor in the AP group was also larger than in the reference group but there was no difference of statistical significance.

Tabl IV Women hospitalized for MI women with AP and a reference group of women aged 54 classified according to number of stressors (%)

Number of stressor	Married women			Single women		
	MI	AP	Reference group	MI	AP	Reference group
	n 37 ^a	n 16	n 67	n 5 ^b	n 4	n 23
0	43	38	54	60	75	11
1	27	50	30	20	25	22
2	16	12	12	20	0	0
3	11	0	4			
4	0	0	0			
5	3	0	0			

^aData on 2 women missing ^bData on 3 women missing

Subjective stress experience

A history of psychological stress according to the women subjective feeling (2.6 in Table V) was significantly more often reported among the women with MI and AP than in the reference group. "Severe stress" (5.6 in Table V) was also significantly more common in the MI group (31 %) and in the AP group (21 %) when compared to the reference group (7 %). The women with coronary ECG did not differ significantly from the women in the reference group.

Tabl V Stress experience in women hospitalized for MI in women with AP and coronary ECG and in a reference group of women aged 50 and 54

Stress experience	MI	AP	Coronary ECG	Reference group
	n 42 ^a	n 28 ^b	n 23	n 578
1 Never	25	29	52	51
Occasionally	14	14	13	16
3 Occasionally during the last five years	14	21	17	13
4 Several times during the last five years	17	*** 14	** 13	NS 13
5 Continuously during the last year	6	*** 7	0	3
6 Continuously during the last five years	25	14	4	4

** p < 0.01 *** p < 0.001 significantly different from women in the general population † S No statistical significance

^aData on 5 women missing ^bData on one woman missing

Personality traits

The aim was chiefly to test the hypothesis that the coronary prone behavioural pattern Type A as described by Friedman and Rosenman^{65 156} was over-represented in the women with MI. The two personality traits studied in the present material which are considered to measure this behavioural pattern (aggression and achievement) tended to score higher in the MI group than in the reference group. The difference was significant for aggression ($p < 0.05$) but not for achievement. Among the other personality traits tested (Table VI) only the factor neurotic self-assertiveness scored significantly higher in the MI group than in the reference group ($p < 0.05$). None of the personality traits scored significantly higher in the AP group than in the reference group.

Smokers in the reference group tended to score higher for aggression than non-smokers (mean scores 4.4 and 3.2, S.D. 3.1 and 2.3 respectively, $p < 0.10$). Mean scores for achievement were similar in smokers and non-smokers in the reference group (6.8 and 6.3, S.D. 2.6 and 3.0).

Table VI. Personality traits (scores) in women who had been hospitalized for MI and in a reference group of women aged 54.

Personality trait	Women with MI n = 42 ^a		Reference group n = 68		Significance of difference
	Mean	S.D.	Mean	S.D.	
Achievement	7.1	3.0	6.5	2.8	NS
Aggression	4.9	2.7	3.7	2.7	$p < 0.05$
Dominance	6.9	3.7	6.4	3.5	NS
Affiliation	10.1	2.5	9.2	2.9	NS
Defence of status	7.8	3.3	7.3	2.9	NS
Guilt feelings	8.8	3.0	7.7	3.1	NS
Exhibition	4.6	3.3	4.3	3.0	NS
Autonomy	7.3	1	7.3	2.3	NS
Nurturance	11.6	2.2	12.1	1.9	NS
Order	11.9	2.7	11.9	2.4	NS
Success	8.1	2.6	7.8	2.8	NS
Neurotic self-assertiveness	146.3	34.7	128.1	36.3	$p < 0.05$
Rational dominance	6.0	60.3	7.9	53.1	NS
Aggressive non-conformance	3.4	27.4	7.6	2.4	NS
Passive dependency	12.9	30.7	114.8	31.6	NS
Sociability	73.9	16.5	77.1	17.4	NS

NS: No statistical significance.

^aData on 5 women missing.

DISCUSSION

High socio economic status has usually been considered to be associated with IHD in men but the opposite has also been reported as reviewed by Lehman¹⁰⁹ and Jenkin⁸⁴. In a previous study in Göteborg⁵ the incomes of men with MI tended to be lower than those of their controls. A similar tendency was found in the present study of women with MI. The incomes of their husbands were about the same in the IHD groups and the reference group in the present study. The lower incomes of the women with IHD may partly be explained by pre-existing heart disease which may have forced them to stop working. Being pensioned was more common in the women with MI than in the reference group. Similarly their husbands were more often unemployed.

Men born outside the Göteborg area were significantly over-represented among men with MI in Göteborg⁵. This was not found for women with MI in the present study.

Shekell et al.¹⁶⁴ amongst other discussed incongruity of social status between husband and wife as a factor associated with IHD. With the definition of status incongruity used in the present study no significant differences were found between women with IHD and women in the general population.

MI and AP tended to be more common in married than in unmarried women in Göteborg which is in agreement with previous observation¹⁷⁷. A high incidence of IHD in women with many children may be an effect of a large number of pregnancies¹¹ but stress experienced by a mother of many children may also be an explanation.

Psychological stress has been considered to be a common feature in subjects with MI both when measured as the subjective experience of stress⁸⁷ and when measured as the exposure to stressors e.g. defined social events or problems at home or at work¹⁷⁶. In the present study both methods of measurement were used and the reporting of stress may have been biased by the MI attack per se. It is reasonable to suppose that the woman's report of defined stress factors is less influenced by the MI attack than her report of stress experience. It is therefore of interest that both methods gave the same results in the present study. Experience of stress as well as exposure to certain defined stressors was more common in women with MI than in the general population of women. Women with AP who were participating in the population study reported stress more often than the rest of the population sample which is of special interest as the two groups were examined under the same conditions.

All stressors studied tended to be more common in the women with MI than in the reference group except for the stressor number 7 (ill child less than 15 years old). This discrepancy is explained by the fact that the reference group consisted of 54 years old women while there was a range of 40-58 years in the MI group.

In the present study the last child usually left home when its mother was about 54 years of age. If this stressor had been excluded the differences between the women with MI and the reference group would therefore have been even more prominent. The exposure to other stressors studied increased or decreased continuously or was constant with age.

Factors such as explosive temperament and a high degree of occupational leadership seem to be common in men with MI.⁶² The best documented personality trait or behavioural pattern associated with IHD so far seems to be the behavioural pattern Type A. This pattern has been found to be associated with IHD both in retrospective⁶³ and prospective¹³⁷ studies in men and in a retrospective study in women.¹³⁶ The use of psychological standard inventories (Minnesota Multiphasic Personality Inventory and Sixteen Personality Factor Test) has failed to show consistent associations between IHD and other personality traits.¹³ In the present study another standard inventory (CMPS) revealed an increased aggression score in women with MI. It is not known why achievement did not score significantly higher in the MI group as was hypothesised. However, it is well known that women generally acquire lower scores for this trait than men do.³⁸ It is possible that high achievement is also less common in women with MI than in men with MI.

A somewhat unexpected finding was that neurotic self assertiveness was clearly distinguished between the MI group and the reference group. However, this is not inconsistent with a behavioural pattern Type A in MI-prone individuals. It is possible that a tendency to defence of status and guilt feelings in indicating a type of ego weakness is just as important as the more overt behaviour Type A in describing the psychology of the coronary-prone woman. It can be speculated whether the type of psychic vulnerability revealed by high scores on neurotic self assertiveness is not even more fundamental than the behavioural pattern Type A, the latter thus being viewed as a compensatory reaction in a complicated personality.

The behavioural pattern Type A has been found to be associated with factors such as hypertension, hypercholesterolemia and smoking.^{63,136} In a previous study on men in Göteborg smokers scored higher for aggression than non-smokers did.¹³⁷ Men who smoke without known IHD had aggression scores similar to those of men who had MI.⁴ In the present series of male smokers in the reference group tended to score higher for aggression than female non-smokers did and had scores for aggression similar to those of the women who had suffered MI. The findings of the present and previous studies^{63,136} might suggest a central position for behavioural traits in the development of IHD.

PHYSICAL ACTIVITY IN A POPULATION SAMPLE OF WOMEN AND IN WOMEN WITH ISCHAEMIC HEART DISEASE

Call Bengtson

Abstract Physical activity both at work and during leisure decreased with age as found from a population study of women. Physical inactivity both at work and during leisure had been significantly more common during the age period 20-38 in women who later had an acute myocardial infarct than in women in the general population. These results were found when comparing later period of life. A similar tendency was noted in women with angina pectoris although the differences more likely did not reach significant levels.

Physical inactivity has been considered as one of the risk factors for ischaemic heart disease (IHD) ^{67, 168}. In the present chapter the physical activity in a population sample of women is compared with the physical activity in women with angina pectoris (AP) with ECG changes suggestive of IHD (coronary ECG) and in women who had an acute myocardial infarction (MI).

MATERIAL

Reference group The reference group in the present chapter consisted of 578 women aged 50 and 54 who were participants in a population study in Göteborg, Sweden ^{1, 5} and who had about the same mean age as the women in the IHD group ¹. In addition a description is given of the physical activity of the participants in all the age groups studied.

Women with AP Twenty-nine women participating in the population study were found to suffer from AP as defined according to Ro ³⁵.

Women with coronary ECG Twenty-three women had ECG changes suggestive of coronary ECG ¹.

Women with MI Forty-seven women in Göteborg with an acute MI during the years 1968-1970 survived on survival in hospital on the day of the MI group ^{1, 9}. Women who died from MI outside hospital were not included. No valid information could be obtained concerning the previous physical activity.

METHODS

Interview about physical activity The subjects were interviewed about physical activity at work and during leisure time. The main physical activity was the total population sample including those with AP and coronary ECG. The women with MI were interviewed by one of the physicians. According to the extent of physical activity the women were classified into group I-IV. The modification for women of the classification has only been used for men in Göteborg ^{1, 9}. For example, group I for physical activity

at work were assigned women with light office work and no domestic work group II shop work light industrial work or domestic work including the care of one child group III might include hospital work or domestic work including the care of two or three children and group IV heavy work together with domestic work or just domestic work including the care of four or more children. As to activity in leisure time, examples for group I might be reading or looking at television for group II activity such as walking or cycling for half an hour a day for group III regular activity such as running tennis swimming and for group IV heavy activity or competitive sports.

Statistical methods The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation.

Further details of material and methods are given in Chapter I.

RESULTS

Table I classifies the women participating in the population study according to age and activity group at work and during leisure. It will be seen that physical activity diminishes with age. This is more obvious for physical activity at work than for physical activity during leisure. For the same period of life for instance their physical activity when they had been between 20 and 38 years of age no differences were found between the women in the different age groups.

Table I Women in different age groups classified according to physical activity (%)

Activity group	During age 20-38					After age 38					During last year				
	Age group					Age group					Age group				
	38	46	50	54	60	38	46	50	54	60	38	46	50	54	60
Physical activity at work															
I	2	2	1	1	0	2	2	2	1		3	5	3	6	13
II	41	44	38	40	46	60	58	62	73		56	68	69	73	75
III	55	53	60	59	54	37	39	37	26		38	27	28	20	11
IV	2	2	1	0	0	1	1	0	0		3	1	1	1	0
Physical activity during leisure															
I	10	6	5	3	2	10	12	7	10		17	19	18	13	32
II	78	79	76	83	84	77	73	84	81		72	70	67	80	64
III	12	14	19	13	14	13	15	8	9		10	11	15	7	6
IV	0	1	0	1	0	0	0	0	0		1	0	0	0	0

In Tabl II women with various manifestation of IHD are compared to women in the population sample aged 50 and 54. A significantly larger number of women in the MI group than in the general population were classified into activity group I for both physical activity at work and during leisure during all the life periods studied. There was no significant difference in physical activity at work during the age period before 38 between women with AP and women in the general population while women with sedentary work after the age of 38 were over-represented in the AP group. Women who were classified into activity group I or II for activity during leisure during the age period 20-38 were over-represented in the AP group ($p < 0.05$). The physical activity of women with coronary ECG had been similar to that of women in the general population before the age of 38. A significantly larger number of women with coronary

Table II Women with MI (n = 40)^a, AP (n = 29) and coronary ECG (n = 23) and a reference group of women aged 50 and 54 (n = 578) classified according to physical activity (%)

Activity group	Physical activity at work				Physical activity during leisure			
	MI	AP	Coronary ECG	Reference group	MI	AP	Coronary ECG	Reference group
During age 20-38								
I	8***	4	0	1	25***	10	4	4
II	69	31	43	39	58	86	87	78
III	22	62	57	59	17	3	9	18
IV	0	3	0	1	0	0	0	0
After age 38								
I	17***	7*	0	2	31***	17	32***	10
II	69	59	64	59	64	79	65	77
III	14	34	36	38	6	3	4	13
IV	0	0	0	1	0	0	0	0
During lifetime								
I	22***	14**	26***	4	45***	21	39*	16
II	62	69	48	71	50	76	57	71
III	15	17	26	25	5	3	9	13
IV	0	0	0	0	0	0	0	0

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ significantly different from the women in the general population

^aData on 7 women missing

ECG were classified into aativity group I for physical inactivity during leisure after the age of 38 and both at work and during leisure for physical activity during the year previous to the study

DISCUSSION

IHD has been found to be a common feature in persons with sedentary work and in persons with low physical activity in leisure time^{120 127 163 184} Most studies have been performed on men but physical inactivity seems to be associated with an increased frequency of IHD in women also¹²⁰

Low physical activity both at work and in leisure time was more often noted in the women who had MI than in the women in the general population in the present study. A significantly larger number of women with low activity during leisure during the age period 20-38 and at work after that age period was not found among the women with AP. The physical activity during the age period 20-38 was similar in the women with coronary ECG and in the reference group of women but lower in the women with coronary ECG during the year previous to the study.

Conclusion must be drawn with caution as interobserver variation may have influenced the result. It is also reasonable that women with AP during the study because of chest pain. The significantly larger number of women with low physical activity at work during the year previous to the study but not during the age period 20-38 years in the AP group may indicate this. As an attack of MI is often preceded by a period of AP¹⁷ effort pain may have reduced the physical activity during a period before the MI occurred. It is therefore important to note that significant difference in physical activity between women with MI and women with AP on one hand and women in the general population on the other was found already at an early age. This was not found in the women with coronary ECG. The observation concerning the age period 20-38 favours the view that physical inactivity is associated with IHD in women. It is true that there was no association between low physical activity at an early age and coronary ECG in women but it is doubtful whether coronary ECG is defined as a manifestation of IHD in women.^{1 11}

Chapter XII

PREVALENCE OF MULTIPLE RISK FACTORS FOR ISCHAEMIC HEART DISEASE IN WOMEN WITH AND WITHOUT KNOWN ISCHAEMIC HEART DISEASE

Calle Bengtsson

Abstract A large number of co-existing risk factors for ischaemic heart disease was found in women who had suffered myocardial infarction than in women in the general population. Similar observations were made when comparing women who had angina pectoris to women who had ECG changes suggestive of ischaemic heart disease with women in the general population though the differences were small. Diabetes mellitus, smoking and high triglyceride were particularly common in women who had had myocardial infarction. At least one of these risk factors was present in 95 % of these women compared to 48 % of the women with angina pectoris, 43 % of women with ECG changes suggestive of ischaemic heart disease and 47 % of the women in the general population. It seems that the prevalence of a large number of risk factors even though the factors may differ only moderately from those usually found in the population, per se acts as an important risk factor for ischaemic heart disease in women.

Conclusions on ruling risk factors for ischaemic heart disease (IHD) have usually been based upon studies of men. The prevalence of a large number of co-existing risk factors which have been found to be statistically associated with IHD has been found to be a common feature in men prone to IHD.^{1,5,7} The present chapter aims to study the co-existence of several risk factors in the individual subject in order to see whether an increased number of these is associated with an increased risk of IHD in women also and if the coronary prone woman has a peculiar combination of risk factors.

MATERIAL

Reference group The reference group in the present chapter consisted of 578 women aged 50 and 54 who were participants in a population study in Göteborg, Sweden^{1,19} and who had approximately the same mean age as the women with IHD.¹

Women with angina pectoris Twenty-nine women participating in the population study were found to suffer from angina pectoris (AP) as defined according to Rose.⁵

Women with coronary ECG ECG changes defined as coronary ECG¹ were recorded in 23 women participating in the population study.

Women with myocardial infarction All people in Göteborg born in 1913 or later who have had an acute attack of myocardial infarction (MI) have been registered in the list of January 1968.^{1,20} Forty-seven women who were survived on arrival in hospital were registered during the year 1968-1970. Another 14 women died from a probable IHD attack outside hospital.

or while hospitalized for reasons other than suspected MI^{VI} 10 of whom were classified as deaths from MI^I

METHODS

The methods used have been described previously in connection with the analysis of the separate risk factors^{I, K-IV}. In the present chapter the following definition of risk levels have been used

- 1) blood pressure for the women participating in the population study this means those on antihypertensive treatment or those who had a systolic blood pressure value in the seated position within the upper two deciles for the women with MI this means those on antihypertensive treatment or those who had a systolic blood pressure value in the seated position within the upper two deciles of the former female group the blood pressure being measured at the 3 month control or at the 12 month control^I
- 2) cholesterol for the women participating in the population study this means a cholesterol value within the upper two deciles for the women with MI this means a cholesterol value within the upper two deciles of the former female group after adjustment for the difference in the serum lipid determinations the cholesterol value being measured either at the time of the acute attack at the 3 month control or at the 12 month control^{VI} for those who died from an acute attack of MI this means that dietary restrictions and/or lipid reducing drugs had been prescribed because of a high serum cholesterol value
- 3) triglycerides as for cholesterol for women participating in the population study and for MI survivors for those who died from an acute attack of MI this means that dietary restrictions and/or lipid reducing drugs had been prescribed because of a high serum triglyceride value
- 4) smoking ≥ 1 cigarette per day
- 5) diabetes history of clinical diabetes mellitus or clinically manifest diabetes mellitus at the population study examination or in association with the acute attack of MI
- 6) pregnant ≥ 4 pregnancies
- 7) menopause menopause at the age of 45 or earlier
- 8) psychological three periods of stress experience for a month or longer during the last 5 years which included anxiety nervousness fear and sleeplessness in connection with conflicts^I
- 9) overweight average body weight⁶⁰ $\geq 10\%$
- 10) physical inactivity two or ≤ 5 scale points when summarizing scale points from the age periods (20-38 after 38 and the year preceding the population study = the MI attack) according to a scale similar to one previously applied to men Gotberg^{3, 34} but modified for women in the present study

meaning work mainly in the e at d position^{II}
 ii) physical inactivity during leisure time & 5 scale points when summarizing
 scale points^{201 204} from the age periods (20-38 after 38 and the year pe
 ending the population study = the MI attack) meaning mostly reading and
 looking at television^{II}

Statistical methods

The hypothesis of difference in frequency of number of risk factors between groups was tested by means of the binomial distribution with a normal approximation. The hypothesis of difference in frequency of the parat risk factors was also tested in the same way mainly in order to obtain some information about their importance as predictor of IHD.

Further details of material and method given in Chapter I.

RESULTS

The prevalence of parat risk factors presented individually for each woman who had suffered MI is shown in Table I. The number of risk factors in the individual subjects is presented in Table II and visualized in Figure 1.

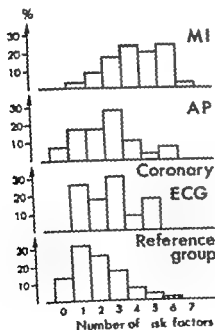


Fig 1 Number of retained clinical risk factors in women with MI (n = 34) (those with < 2 missing data are omitted) in women with AP (n = 29) in women with Coronary ECG (n = 23) and in women in the reference group (n = 578).

Table I Prevalence of certain defined risk factors (x) in the individual women in the MI groups I high BP II high serum cholesterol III high serum triglycerids IV smoking V diabetes mellitus VI ≥ 4 pregnancies VII premature menopause VIII = psychological stress IX overweight X = physical inactivity at work XI physical inactivity during leisure time MD missing data

Subj no	Age (years)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Hospitalized because of MI survivors on arrival in hospital												
1	37	x	x			x			MD		MD	MD
2	43		x	x	x				x	MD		
3	44		x		x				x		x	x
4	44	MD	MD	MD		x	MD		MD	x	x	x
5	45			x	x	x	x		x			x
6	46	x		x	x						MD	MD
7	47			MD	x				MD			MD
8	47			x	x						x	x
9	48	x	x		x				x	x		
10	48							x	x			
11	48	x	x	x	x					x		
12	48	x		x	x		x			x		
13	49	x		x	x		x					
14	49		x		x							x
15	49			x		x		x		x		
16	51	x		x	x				x	x	x	
17	51	MD	MD	MD						MD	x	x
18 ^a	51	MD		x	MD	x	MD	MD	MD	MD	MD	MD
19	52	x	x	x	x		x					x
20	52		x	x	x				MD	MD	MD	MD
21	52								x			
22	53		MD	MD	x		x		MD	x	MD	MD
23	53	x	x	x	x			x	MD		MD	MD
24	53	x		x	x					x		
25	53	x		x	x			x	x			
26	53	x		x	x			x		x	MD	x
27	54			x	x	x		x	x	x		
28	54	x		x	x		x		x			x
29	54	MD	MD	MD	x		MD		MD	MD	MD	MD
30 ^a	54	x	x	x	x	x			MD		MD	MD
31	55	x	x		x							
32	55				x		x	x	MD		MD	MD
33	55	x		x	x		x	x	x			
34	55			x	x			x				
35	55		x	x	x						x	
36	56	x			x							x
37	56	x		x	x		x		x			x
38	56			x	x			x				
39	56	x		x			x		x	x	x	
40	56				x				x			
41	56	x		x	x		x		x	x		x
42	56	x		x	x				x			x
43 ^a	56	x	x	MD					MD		MD	MD
44	57	x			x							
45	57			x	x				x			
46	57	x		x	x				x			x
47	57	x		x	x					x		

^a Died during the period of hospitalization

Table I (contin)

Sub ject no	Age (year)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
MI deaths outside hospital or while hospitalized for reasons other than MI												
1	40		MD	MD	MD			MD	MD	MD	MD	MD
	42	x	x	MD	MD		x	MD	MD	MD	MD	MD
3	48	x		MD				MD	MD	MD	MD	MD
4	51	MD	MD	MD		x			MD	MD	MD	MD
5	51	x	x	MD	x				MD	MD	MD	MD
6	51	MD	MD	MD	MD		MD	MD	MD	MD	MD	MD
7	53	x			MD			MD	MD	MD	MD	MD
8	54	x	x	x	MD	x	x		MD	MD	MD	MD
9	54	x			MD			x	MD	MD	MD	MD
10	56	MD	MD	MD	MD		MD	MD	MD	MD	MD	MD

Women with MI as well as women with AP and coronary ECG usually had a larger number of risk factors than women in the general population. The distribution of risk factors was skewed to the right in the MI group (Fig 1) and to the left in the reference group while the groups of women with AP and coronary ECG showed an intermediate distribution. All women with MI had at least 1 risk factor (Table II) compared to 93 % of the women with AP, 100 % of the women with coronary ECG and 86 % in the reference group. 97 % in the MI group had at least 2 risk factors as compared to 76 % in the AP group, 74 % in the coronary ECG group and 55 % in the reference group. The proportion of women with a certain minimum number of risk factors was significantly greater in the MI group than in the reference group. Similar tendencies were found for the women with AP and for the women with coronary ECG though all the differences did not reach significant levels. It is also shown in Table II that none of the women in the various groups had more than 7 of the 11 risk factors studied.

The data of Table II and Fig 1 concern 34 women who had had MI and in whom data concerning none or at most one variable was missing. Also from Table I multiple risk factors were also common in the remaining 13 women for whom a larger number of data was missing and in the women who died from a MI attack without being hospitalized for suspected MI.

In Table III the parallel risk factors are ranked according to the χ^2 value obtained when comparing the prevalence of these risk factors in the group of women with IHD with the prevalence in the reference group. This method of ranking is to be regarded only as an attempt to obtain some information about the importance of the parallel risk factors. The highest value in the MI group was recorded for serum triglyceride, diabetes mellitus and smoking in the AP group for previous physical activity at work, many pregnancies and psychological stress and in the group of women with coronary ECG for high BP.

Table II Women with MI AP and coronary ECG and women in the reference group tabulated according to individual number of retained risk factors^a (%)

Number of risk factors	Women with MI n = 34 ^a	Women with AP n = 29	Women with coronary ECG n = 23	Reference group n = 578
0	0	7	0	14
≥ 1	100**	93	100*	86
≥ 2	97***	76*	74*	55
≥ 3	88***	59***	57**	49
≥ 4	71***	41	26*	12
≥ 5	47***	10	17*	5
≥ 6	26***	7*	0	1
≥ 7	3***	0	0	0

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ significantly different from women in the general population

^a 13 women with ≥ 2 missing data omitted

physical inactivity during leisure and physical inactivity at work. These values were consistently lower for the women with AP and coronary ECG than for the women in the MI group.

At least one of three factors: diabetes mellitus, smoking, or serum triglyceride values which were within the upper quintile of the population sample was recorded in 38 of 40 women with MI (95%) for whom data on all three variables were available. This is to be compared to 14 of 29 women with AP (48%), 10 of 23 women with coronary ECG (43%) and 273 of 575 women in the population sample (47%). Thirty-seven of 40 women who had had MI (93%) were smokers and/or had elevated serum triglyceride values compared to 48% of the women with AP, 39% of the women with coronary ECG, and 47% of the women in the population sample. Both smoking and elevated serum triglyceride levels were recorded in 68% of the women with MI, in 10% of the women with AP, 9% of the women with coronary ECG, and in 10% of the women in the population sample.

Table III Ranking of values when comparing the prevalences of certain risk factor in women with IHD to those of the women in the reference group

Factor studied	value	Statistical significance
<u>Women with MI</u>		
High serum triglyceride	7.70	$p < 0.001$
Diabetes mellitus	6.40	$p < 0.001$
Smoking	5.67	$p < 0.001$
Physical inactivity at work	5.64	$p < 0.001$
High blood pressure	5.32	$p < 0.001$
Psychological stress	3.87	$p < 0.001$
Physical inactivity during leisure	3.79	$p < 0.001$
Premature menopause	2.01	$p < 0.05$
≥ 4 pregnancies	1.60	($p < 0.10$)
High serum cholesterol	1.28	NS
Overweight	1.23	NS
<u>Women with AP</u>		
Physical inactivity at work	3.94	$p < 0.001$
≥ 4 pregnancies	2.83	$p < 0.01$
Psychological stress	1.99	$p < 0.05$
High blood pressure	1.62	($p < 0.10$)
High serum triglyceride	1.43	($p < 0.10$)
Physical inactivity during leisure	1.33	($p < 0.10$)
Premature menopause	1.03	NS
Overweight	0.63	NS
High serum cholesterol	(0.12) ^a	NS
Diabetes mellitus	(0.56) ^a	NS
Smoking	(1.12) ^a	NS
<u>Women with coronary ECG</u>		
High blood pressure	3.91	$p < 0.001$
Physical inactivity during leisure	2.61	$p < 0.01$
Physical inactivity at work	2.04	$p < 0.05$
High serum triglyceride	1.70	$p < 0.05$
Premature menopause	1.58	($p < 0.10$)
Diabetes mellitus	1.44	($p < 0.10$)
≥ 4 pregnancies	1.32	($p < 0.10$)
Overweight	0.83	NS
High serum cholesterol	0.52	NS
Psychological stress	(0.28) ^a	NS
Smoking	(2.43) ^a	NS

^aMissing in the reference group NS No statistical significance

DISCUSSION

With the definition of risk level used in the present paper significant as almost significant differences were found between the women with MI and the women in the reference group for all the variables studied but two serum cholesterol and body weight. In spite of this high serum cholesterol value and overweight have been included in the present analysis as they are usually

taken into consideration when discussing risk factors

The risk levels were arbitrarily chosen but were usually the same in the subjects with IHD as in the reference group. Direct comparison is not always possible. For instance, the blood pressure is known to fall in connection with a MI attack^{136, 102} and the blood pressure measured in association with and after a MI can therefore not be considered as valid for the pre-infarction blood pressure of the subject. The highest blood pressure of those which were recorded either at the 3 month control or at the 12 month control was therefore related to the casual blood pressure of those participating in the population study. In addition, previous antihypertensive treatment was used as a complementary criterion. This complementary criterion is unbiased by the MI attack, but it is possible that more subjects with a high blood pressure were on treatment in the MI group as those who had a MI may have visited a doctor more often because of AP or other symptoms or diseases which were overrepresented in the women with MI.¹⁷ For this reason antihypertensive treatment was not used as a single criterion for risk level of blood pressure. Nor was the definition exactly the same for the women with MI and the women in the population sample when serum cholesterol and serum triglycerides were studied. The casual serum lipid values were used for comparison in the population sample while in the women who had MI the highest of the values measured either at the time of the acute MI attack at the 3 month control after the attack or at the 12 month control was used. The decrease in serum lipids known to occur in connection with a MI attack^{67, 107, 102} is thereby considered to be compensated.

The results obtained in the present study agree with those of a previous study of men in Göteborg¹³⁴ which showed that the risk of MI and AP increased with increasing number of risk factors. The combination of high blood pressure, high serum cholesterol and cigarette smoking has been considered to be of particular importance as predictors of MI in men.^{34, 34, 44, 104} Less is known about the importance of the separate risk factors for IHD in women. Judging from the present study of women, high serum triglycerides, diabetes mellitus and smoking are particularly overrepresented in women with MI. It is to be noted that at least one of the three risk factors was present in 95 % of the women who had had MI. It thus seems that some separate factors are more overrepresented than others in women who have MI, but it is evident that the risk of MI increases with an increasing number of risk factors present.

The risk factor pattern of the women with AP seemed to be different from that of the women with MI. For example, smoking was much less common in the women with AP than in the women with MI. The combination of smoking and high serum triglycerides which was so commonly registered in the women with MI was no more common in the women with AP than in the population sample. However, a large number of risk factors than the population sample was

found in the women with AP also

The number of risk factors were about the same in the women with coronary ECG as in the women with AP but the "risk factor pattern seemed to be different from that of the AP group and also from that of the MI group. A high blood pressure was a particularly common feature in the women with coronary ECG which has brought into consideration the possibility that the coronary ECG as defined in the present study might be a manifestation of hypertension rather than IHD in women¹

The present results seem clear in spite of methodological difficulties. The individual factors for the women are perhaps only slightly different from those of the women in the general population but even moderate differences seem to be important when a number of them are present. It seems that a number of such moderate differences per se is an important risk factor for the development of MI in women.

taken into consideration when discussing risk factor

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The risk factor pattern of the women with AP seemed to be different from that of the women with MI. For example, smoking was much less common in the women with AP than in the women with MI. The combination of smoking and high serum triglycerides which was so commonly registered in the women with MI was no more common in the women with AP than in the population sample. However, a large number of risk factors than in the population sample was

and 180 were aged 54. The ages of the women in the two age strata were identical with those of the men when the men were examined in 1963 and 1967 respectively. Comparisons were made between men and women in these two age groups.

METHODS

The men as well as the women were examined under strictly standardized conditions. With a few exceptions the same techniques and methods were applied. The physical examination of all the men was performed by one physician (G.T.). All the women were also examined by one physician (C.B.). The examination of the men in 1963 and the examination of the women were performed in the morning the subject fasting. In 1967 the men were examined in the afternoon.

Blood pressure determination. The same technique was applied to men and women with the exception that the blood pressure of the men was read to the nearest 5 mm Hg but in women to the nearest 2 mm Hg. Men who were found to have systolic blood pressure ≥ 175 mm Hg and diastolic blood pressure ≥ 115 mm Hg in 1963 received antihypertensive drugs.

Serum lipid. The total cholesterol level of the men was determined according to Cárner and Isaksson⁴⁰. The serum cholesterol levels of the women were determined according to a modification of the method described by Levin and Zak¹¹². The serum triglyceride levels of the men were determined according to Carlson¹² and the triglyceride levels of the women according to a modification of the method described by Loftland¹¹⁵. The methods used in the study of women gave higher values both for cholesterol and triglycerides than those used in the studies of men.¹¹⁶ The examination of the men in 1967 (when the men were aged 54) was not performed in the fasting state. Serum triglyceride was therefore not determined in 1967.

Haematology. Venous blood was withdrawn without anticoagulant with the subject in the supine position. A microhaematocrit centrifuge was used. The mean value of two duplicate readings was used in the calculation.

Smoking habits. Information concerning smoking habits was obtained by interview.¹¹⁷ On average was estimated to be equivalent to 1 g of tobacco 1 cigarillo, 2 g and 1 cigar, 5 g tobacco. Subjects who smoked ≥ 1 g tobacco/day were defined as smokers.

Alcohol consumption. Information concerning alcohol consumption was obtained from 54-year-old men and women by interview. Type of consumption (pirate wine, beer) and frequency (Table III) were recorded.

Diabetes mellitus. Information was obtained by interview. Those with a history of fasting hypoglycaemia and glycosuria were defined as diabetics. In addition, on women with previously unknown diabetes but fasting hypoglycaemia and glycosuria in the population study were considered to have manifest diabetes.

Mental stress Information was obtained by a standardized interview. The subjects were asked whether they had had a feeling of stress for a month or longer including tension, fear, anxiety or disturbances of sleep in connection with conflicts in the family at work etc.¹

Statistical methods Conventional statistical methods were used for calculation of mean values, standard deviation (S.D.) and standard error of the mean (S.E.). Significance of differences between mean values was estimated with Student's *t* test. The hypothesis of difference in frequencies between groups was tested by means of the binomial distribution with a normal approximation (two-tailed *t* test).

Further details of material and method are given in Chapter I and in the descriptions of the population studies of men¹⁰² and women¹⁵.

RESULTS

Arterial blood pressure

Systolic blood pressure was similar in men and women (Table I). There is a tendency towards higher diastolic blood pressure values in men. The difference was, however, slight. The distributions of blood pressure were also similar in the two sexes. This is shown for the systolic blood pressure in 50-year-old men and women in Fig. 1.

Table I. Arterial blood pressure, serum lipids and haematocrit in men and women aged 50 and 54.

Age	Men				Women			
	n	Mean	S.D.	S.E.	n	Mean	S.D.	S.E.
Systolic blood pressure (mm Hg) in the seated position								
50	855	138	20.9	0.71	397	138	21.8	1.09
54	799	144	21.2	0.75	180	143	24.1	1.80
Diastolic blood pressure (mm Hg) in the seated position (Phase 4)								
50	855	92	13.2	0.45	397	88	10.6	0.53
54	799	92	12.2	0.43	180	89	12.1	0.90
Serum cholesterol (mg/100 ml)								
50	855	247	4.9	1.47	398	276	41.9	2.10
54	633	270	46.4	1.84	178	284	41.7	3.13
Serum triglycerides (mmol/l)								
50	855	1.26	0.82	0.03	398	1.26	0.58	0.03
54					178	1.39	0.69	0.05
Haematocrit (%)								
50	855	44.9	3.9	0.11	398	39.7	2.95	0.15
54	796	44.1	3.43	0.12	180	40.0	6.3	0.20

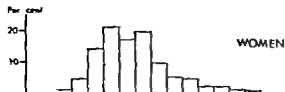
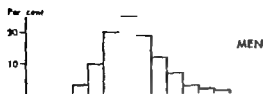


Fig 1 Distribution of systolic blood pressure in 50 year old men and women. The Study of Men Born in 1913 and the Study of Women 1968-1969



80- 100- 120- 140- 160- 180- 200- 220-
mm Hg mm Hg mm Hg mm Hg mm Hg mm Hg mm Hg

In a previous communication¹⁸ it was shown that hypertensive women were more often on antihypertensive treatment than hypertensive men. Thus 4.5% of 50 year old women were on antihypertensive drugs compared to only 1.6% of 50 year old men ($p < 0.05$). Excluding those on antihypertensive drugs 3.9% of the men and 1.1% of the women had systolic blood pressure ≥ 175 mm Hg and diastolic blood pressure ≥ 115 mm Hg.

Serum lipids

Mean of serum total cholesterol and serum triglyceride values for men and women aged 50 and 54 are presented in Table I. After adjustment for the different methods of serum lipid determination in the two studies¹⁹ the mean of serum total cholesterol was found to be 17 mg/100 ml higher in 50 year old women and 4 mg/100 ml higher in 54 year old women than in men of the same age while the mean of serum triglyceride was found to be 0.28 mmol/l higher in 50 year old men than in 50 year old women.

Hematocrit

Means of hematocrit values in men and women aged 50 and 54 are shown in Table I. The hematocrit value of the men was strikingly higher than that of the women ($p < 0.001$).

Smoking habits

There were more smokers and more ex-smokers among the men (71%) than among the women (41%) ($p < 0.001$). Smoking at least 10 cigarettes per day was also significantly more common in men than in women (11% vs 3%) ($p < 0.001$). Among the smoking in the general population ($p < 0.001$) for the group 50 ($p < 0.01$) for the group 54).

Table II Smoking habits in 855 men and 398 women aged 50 and in 803 men and 180 women aged 54 (% M men F women)
The Study of Men Born in 1913 and the Study of Women 1968-1969

Age (years)	Smokers		Ex smokers		Non smokers ^a		Smoking ^b ≥ 15 g/day	
	M	F	M	F	M	F	M	F
50	56	37	20	6	24	57	20	7
54	54	38	26	6	20	56	17	8

^aEx smokers not included ^bOf the total sample

Alcohol consumption

Men consumed alcohol more often than women did. The difference was statistically significant for spirits and beer (Table III) while wine was consumed as often by women as by men.

Table III Alcohol consumption in 803 men (M) and 180 women (F) aged 54 (%)

Frequency	Spirits		Wine		Beer	
	M	F	M	F	M	F
Every day	3***	1	1 NS	1	36 **	14
Several times a week	14	2	5	4	22	14
Once a week	28	~	13	12	15	22
Once a month	22	18	19	36	6	16
Seldom or never	32	73	61	47	0	34

*** $p < 0.001$ significantly different from women NS No statistical significance

Diabetes mellitus

The prevalence of diabetes mellitus was similar in 50 year old men and women (0.8% in the men and 1.3% in the women) and in 54 year old men and women (1.0% and 0.6% respectively).

Mental stress

When interviewed in the same standardized way 54 year old men reported continuous experience of mental stress during the year previous to the study more often than 54 year old women (18% as compared to 7% $p < 0.001$).

DISCUSSION

According to results obtained in previous studies^{47, 50, 51} blood pressure has been found to be higher in men up to the age of about 50 and then higher in women but with no marked differences at any age. Blood pressure in men and women aged 50 and 54 may thus be expected to be about the same and this was also found in the Göteborg studies. The slight difference noted for diastolic blood pressure may be due to inter-observer variation. Statistical analysis has not been performed for blood pressure as the conditions were not exactly the same. However the results are clear enough to show that there are no important differences in blood pressure between men and women at the ages which can explain a higher incidence of MI in men. It is possible that the slightly higher blood pressure in men than in women of younger age may contribute to the sex difference in MI but the difference is probably too small to be of importance.

Women were more often on antihypertensive treatment than men were. Possibly part of this difference may be explained by the time lapse between the studies of men and women. However if this really is a difference this can probably not explain much of the sex difference in MI, especially as the age and distribution of blood pressure were similar in spite of the difference in number on antihypertensive treatment.

A different method for determination of total cholesterol and triglyceride levels had been used for men and women. Statistical analysis was not performed and conclusions from comparisons between the two sexes in this respect must be drawn with caution. After adjustment for difference in method of serum lipid determination, serum cholesterol was found to be about 5.14 mmol/100 ml higher in women than in men in the age studies while serum triglyceride were about 0.30 mmol/l higher in the men. These sex differences agree with the noted in Stockholm Study⁵⁴. Thus there are probably no differences in serum lipid at the age which can explain the sex difference in MI. Serum cholesterol levels have been found to be higher in men than in women up to the age of about 50 and then higher in women while serum triglyceride levels seem to be higher in men both before and after the age of 50^{54, 55}. The high cholesterol and triglyceride values in men before the age of 50 may contribute to the difference in incidence of MI between the sexes but as for blood pressure the sex difference is in younger age probably too slight to be of great importance.

The haematocrit values were much higher in men than in women. A high haemoglobin value has been discussed as a risk factor for IHD in men^{56, 57}. A multivariate analysis of risk factors for IHD in men in Göteborg⁵⁸ did not show a significant relation between haematocrit and MI. Haematocrit was not a risk factor in the present study of women who suffered from MI. Haemoglobin values were not high in women who suffered from MI than in women

in the general population. Although there seem to be no definite association between a high haematocrit value and MI in either sex, the difference in haematocrit between the sexes might explain some of the sex difference in the incidence of MI.

In agreement with previous studies^{22, 48, 97, 129} smoking was much more common in men than in women. And the male smokers were more often heavy smokers. The sex difference in smoking habit may be one reason for the sex difference in MI.

Alcohol has been discussed as another possible risk factor for MI.^{185, 192, 206} In the present study men consumed alcohol more often than women did. High alcohol consumption in men may be one reason for a higher incidence of MI in men.

A similar prevalence of diabetes mellitus in men and women was found in Göteborg. This is in agreement with previous studies⁴⁹ which indicate that the prevalence of diabetes is about the same in the two sexes up to the age of 50. The sex difference in the incidence of MI can thus not be explained by sex difference in the prevalence of manifest diabetes.

Some previous reports have pointed out that diabetes is more often associated with MI in women than in men.^{28, 62, 104} This agrees with the observation in Göteborg. Thus 15% of women hospitalized for an acute MI had manifested diabetes^{viii} compared to 6% of men with MI.²⁰¹ The difference was not statistically significant. Based upon figures for the prevalence of diabetes in men and women with and without a previous MI and on figures for the incidence of MI in the population,ⁱⁱⁱ the incidence of MI in non-diabetic men in Göteborg could be turned out to be about 10 times that in non-diabetic women, while the incidence in male diabetics was found to be about 3 times that in female diabetics. The incidence in diabetic women was found to be about twice that in non-diabetic men.

It is difficult to evaluate the finding that psychological stress was a more common feature in men than in women, as the way of life is so different in the two sexes. The difference in stress experience reported thus need not necessarily mean that men are more exposed to psychological stress than women. However, if there is a real difference this may explain some of the sex difference in the incidence of MI.

Behavioural studies were studied by means of a questionnaire (CMPS) in the present study. Aggression was found to be significantly more common in women who had suffered MI than in the general population of women. The aggression score was higher in a randomized sample of 52-year-old men in Göteborg than in the sample of 54-year-old women (Table IV). Scores for achievement and dominance were also found to be higher in men (Table IV) while other traits studied gave similar results in men and women. Smokers

and non smokers were studied separately as smoking was expected to be higher.⁴ Aggression and achievement are considered to measure the coronaroprone behavioural pattern Type A⁶⁵ as was previously discussed in Chapter X. It is possible that there are sex differences in behavioural traits which might explain some part of the sex differences in the incidence of MI.

Table IV Means (CMPS) for aggression, achievement and dominance in male (M) and female (F) smokers and non smokers

	Smokers		Non smokers	
	M n 8	F n 30	M n 42	F n 36
Aggression	5.2	4.4	3.8	3.2
Achievement	7.4	6.8	7.1	6.3
Dominance	8.5	6.1	7.5	6.7

Taken together, some differences in risk factors were found between men and women. The sex differences in smoking habit, alcohol consumption, haematocrit and probably in stress experience. There might be differences in personality traits. No important differences were found when comparing a number of the risk factors. It is unlikely that sex differences in known risk factors explain more than some part of the differences in the incidence of MI between men and women. Therefore, it is probably some unknown sex-linked factor is in protecting women from MI in young and middle age.

in the general population. Although there seems to be no definite association between a high haematocrit value and MI in either sex, the difference in haematocrit between the sexes might explain some of the sex difference in the incidence of MI.

In agreement with previous studies^{22, 48, 97, 127} smoking was much more common in men than in women and the male smokers were more often heavy smokers. The sex difference in smoking habits may be one reason for the sex difference in MI.

Alcohol has been discussed as another possible risk factor for MI.^{185, 192, 204} In the present study men consumed alcohol more often than women did. Higher alcohol consumption in men may be one reason for a high incidence of MI in men.

A similar prevalence of diabetes mellitus in men and women was found in Göteborg. This is in agreement with previous studies⁶⁹ which indicate that the prevalence of diabetes is about the same in the two sexes up to the age of 50. The sex difference in the incidence of MI can thus not be explained by sex difference in the prevalence of manifest diabetes.

Some previous reports have pointed out that diabetes is more often associated with MI in women than in men.^{88, 64, 104} This agrees with the observation in Göteborg. Thus 15% of women hospitalized for an acute MI had manifest diabetes^{VIII} compared to 6% of men with MI.²⁰¹ The difference was not statistically significant. Based upon figures for the prevalence of diabetes in men and women with and without a previous MI and on figures for the incidence of MI in the population,^{III} the incidence of MI in non-diabetic men in Göteborg could be estimated to be about 10 times that in non-diabetic women, while the incidence in male diabetics was found to be about 3 times that in female diabetics. The incidence in diabetic women was found to be about twice that in non-diabetic men.

It is difficult to evaluate the finding that psychological stress was a more common feature in men than in women, as the way of life is so different in the two sexes. The difference in stress experienced thus need not necessarily mean that men are more exposed to psychological stress than women. However, if there is a real sex difference, this may explain some of the sex difference in the incidence of MI.

Behavioural factors were studied by means of a questionnaire (CMPS) in the present study.^I Aggression was found to be significantly more common in women who had suffered MI than in the general population of women. The aggression score was higher in a random sample of 52-year-old men in Göteborg than in the sample of 54-year-old women (Table IV). Score for achievement motivation also seemed to be high in men (Table IV) while other traits did not give similar conclusions in men and women. Smokers

age 60 with primarily uncomplicated AP died within 8 years³⁰. These seem to be some additional factor which causes MI in men with AP but seldom in women with AP.

Smoking might be such an additional factor. Smoking is more common in men than in women and more common in subjects with MI than in subjects free from MI irrespective of exercise^{VII}. There seems to be no distinct overrepresentation of smokers in subjects with AP of either sex. Consequently it seems that smoking is of no or little importance in the development of AP but is of great importance in the development of MI. This may be another way to explain why the prevalence of AP is about the same in both sexes while MI is much more common in men than in women.

A number of factors were found to be more common in women with IHD than in women in the general population. Most of these factors are summarized in Table I.

Table I. Comparison between women with IHD in the present series and women in the general population concerning the factors studied.

Factor studied	Women with MI	Women with AP	Women with coronary ECG
Previous MI or AP	+		+
Intermittent claudication	+	+	
Gallstones or renal stones	+	+	+
Arterial hypertension	+	(+)	+
Hypertension of the retina			
Triglyceridemia	+	(+)	+
Smoking	+		
Diabetes mellitus	+		
Glucose intolerance			?
Decreased insulin response	(+)		?
Many pregnancies	(+)	+	+
Premature menopause	+	+	+
Social factors			
Mental stress	+	+	
Certain personality traits	+		?
Physical inactivity	+	+	(+)
Overweight			
High haemoglobin value			
Large number of risk factors	+	+	+

+ statistically significant difference (+) statistically insignificant tendency or no tendency noted ? comparative analysis not carried out
^aIn non-diabetic women

Body weight and haemoglobin are included in Table I although they have not been reported separately in previous chapters. Women with AP were slightly heavier than women in the general population apart from this there was no significant difference in body weight or body height. Being overweight was thus not found to be a risk factor for IHD in women in the present study which agrees with the results from Framingham¹⁰⁰. A raised haemoglobin concentration has been found to be associated with IHD in men^{2, 99} but not in women⁹⁹. The haemoglobin concentrations of women with IHD in the present study were no higher than in women in the general population.

No association was found between high serum cholesterol values and IHD in the present study while there was an association between high serum triglycerides and IHD¹¹. There seems to be an association particularly between cholesterol and IHD in men^{71, 106} and between triglycerides and IHD in women. Such a difference between the sexes may partly be explained by hormonal effect on the serum lipids in women. Estrogen decreases the cholesterol level while they may raise the triglyceride level as is noted for instance during pregnancy or oestrogen administration^{2, 11}.

Women who suffered MI were not asked about previous alcohol consumption. High triglyceride values have been reported in alcohol consumers⁹⁹ but the high triglyceride values of the women with IHD in the present study can probably not be explained in this way.

The number of pregnancies and childbirths tended to be greater in women with IHD than in women in the general population¹¹. This may be explained in many ways. Pregnancy may predispose to IHD for instance by increasing the serum triglycerides. As the experience of stress is also associated with IHD and a large number of children may be a stress factor, the stress experience rather than the number of pregnancies might be the risk factor. Unmarried women tended to be underrepresented among the women hospitalized for MI and in the women with AP. This may be explained by a smaller number of pregnancies among these women. Or is a man to be considered a risk factor for IHD in a woman?

If the influence of estrogen on serum lipids is confusing the association between estrogens and IHD even more confusing it seems that treatment with oestrogens or oestrogen administration for other reasons may induce IHD. On the other hand long term administration of estrogen decreases the incidence of MI and the high incidence of MI in women with premature menopause indicates that estrogen also protects from IHD. The possible influence of estrogen on IHD was discussed in Chapter IX. It was concluded that probably that estrogen may be of importance for the development of thromboembolic diseases but given long term protection from IHD maybe by protecting from the occlusions of the coronary arteries. Athroclerosis of

the coronary artery disease has in an autopsy study be found to be more prominent in men than in women¹⁷⁴

It has been much debated whether the smoking is a causative factor for MI or if smoking is linked to some other factor which is the causative one.¹⁷⁵ Among personality traits such as aggression tend to be more common in smokers than in non smokers and more common in subjects with MI than in subjects who had not suffered MI.¹⁷⁶ On the other hand no association was found between striving for manpation and smoking. For example if male smokers worked outside the home no more often than the female non smokers their incomes were similar and they reported previous mental stress with similar frequency.

Many other examples of possible co variation of various risk factors could be stated. However the present report did not aim to discuss such co variation in detail. As has been emphasized¹⁷⁷ risk factors are mere statistical relationships and say nothing concerning causation. More definite information concerning causation can only be obtained from prospective studies with intervention on risk factors.¹⁷⁸

There was also a difference in number of risk factors between the women with MI and the women with AP or coronary ECG which may probably partly be explained by varying accuracy concerning the criteria of these three manifestations of IHD. Probably the number of false positives is larger among the women with AP or coronary ECG than among the women with MI. An admixture of false positives in the groups of women with AP or coronary ECG may have diminished the differences between the women with AP or coronary ECG and the women in the general population. It also seems that women with MI are different from those with AP. Perhaps some factors concerning coronary atherosclerosis are additional factors AP and still others MI.

There must be some reason or reasons for the higher prevalence and incidence of MI in men than in women. Smoking was discussed as a factor which may explain some of the sex difference in MI. However it cannot explain more than part of the difference as shown in Table II. A rough estimate of the incidence of MI in smoking and non smoking men and women has been based upon incidence data^{179,180} data on smoking from the population study of men and women aged

Table II Approximate annual incidence of MI per 1000 smoking and non smoking men and women aged 40-54 in Göteborg

Category	Approximate annual incidence of MI
MI smokers	4.5
MI non smokers	1.0
F male smokers	0.9
F male non smokers	0.1

50 and 54^{III} a previous report on smoking in men with MI⁴⁹ and data concerning smoking in the present study of women with MI^{VII} is presented in this table. MI was more common in male smokers than in female smokers. The incidence was approximately similar in male non smokers and female smokers.

Experience of mental stress was more often reported in men than in women but this difference can probably only explain a small part of the sex difference in the incidence of MI. Men consumed alcohol more often than women did. Haematocrit was higher in men. Serum triglyceride levels seemed to be higher in men than in women both before and after the age of 50^{III & 99}. High serum triglyceride levels were common in women who had suffered MI^{VII III}. A sex difference in serum triglycerides might therefore be of importance. The age trend with higher blood pressure and higher serum cholesterol levels in men than in women up to the age of about 50^{III & 99} may possibly also explain some of the sex difference in the incidence of MI. Otherwise there were no important sex differences in risk factors.

Summarising differences in various risk factors it seems probable that they may explain part of the sex difference in MI. However the most prominent difference between the sexes in this respect is obviously still the simple fact that men are men and women are women.

GENERAL SUMMARY

The present study was based upon a sample of the female population and on a series of women in the same population who suffered myocardial infarction (MI) during a defined period of time. Women in the population sample with symptoms of angina pectoris (AP) and ECG changes suggestive of ischemic heart disease (coronary ECG) were studied separately. Comparisons were made between women with various manifestations of ischemic heart disease (IHD) and women in the population sample who were free from the IHD manifestation studied. A comparison was also made between the male and female populations.

MI was more common in men while the prevalence of AP was similar in men and women. A history of previous MI and/or AP was common in women who suffered MI. Arterial hypertension, high serum triglyceride, smoking, diabetes mellitus, mental stress and physical inactivity were common features in this group of women. Premature menopause was more common than in women in the general population. However, none of the women who had a MI before the age of 45 had reached the menopause at the time of the MI. Coexistence of several risk factor characterized the women with MI.

The characteristics of the women who had AP were similar to those of the women who had MI with the exception of smoking which, in contrast to women with MI, was no more common in women with AP than in those free from AP in the population.

None of the women in the population sample had ECG changes suggesting a previous MI. The ECG changes defined as coronary ECG almost exclusively comprised ST depressions and T inversions. Hypertension was a common feature in the women with coronary ECG which might suggest that coronary ECG as defined in the present study is a manifestation of hypertension rather than IHD in women.

Smoking and alcohol consumption were more common in men than in women. Hematocrit was higher in men. Men more often reported experience of mental stress. However, differences in established risk factors cannot explain more than part of the sex difference in the incidence of MI. There seems to be some other sex-linked factor or factors protecting women from MI in young and middle age.

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Effects of propranolol on hemodynamic parameters and plasma renin activity

By Lennart Hansson

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Beta-Adrenergic Blockade in Essential Hypertension

Effects of propranolol on hemodynamic parameters
and plasma renin activity

By
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GÖTEBORG 1973

This study is based on the following papers.

- I Hansson, L. & Zweifler A.J The effect of propranolol on plasma renin activity and blood pressure in essential hypertension.
- II Hansson, L., Zweifler A.J Julius, S & Hunyor S.N Hemodynamic effects of acute and chronic beta-adrenergic blockade in essential hypertension.
- III Hansson, L & Zweifler A.J Correlation of the degree of beta-adrenergic blockade to plasma propranolol concentrations in the treatment of hypertension. The clinical usefulness of the nitroglycerine test.
- IV Hansson, L Zweifler A.J Julius, S & Ellis, C.N.. Propranolol therapy in essential hypertension. Observations on predictability

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ABBREVIATIONS AND NOMENCLATURE

BSA	= Body Surface Area
HR	= Heart Rate
LVH	= Left Ventricular Hypertrophy
MAP	= Mean Arterial Pressure
n.s.	= Not significant
PRA	= Plasma Renin Activity
Q	= Cardiac Output
Qi	= Cardiac Index
SV	= Stroke Volume
SVI	= Stroke Volume Index
TPR	= Total Peripheral Resistance
TPRi	= Total Peripheral Resistance Index

The somewhat controversial expression "essential hypertension" has been used throughout this paper to indicate primary hypertension, i.e. extensive clinical and laboratory studies have not revealed the etiology of the high blood pressure. Furthermore, "propranolol" is used for di-propranolol chloride.

INTRODUCTION

The present study is concerned with the effects of acute and prolonged beta-adrenergic blockade in essential hypertension. Special interest has been devoted to hemodynamic alterations

and changes of plasma renin activity seen after acute intravenous administration of propranolol and following four weeks of oral treatment.

Hemodynamics in essential hypertension

It is generally agreed that total peripheral vascular resistance at rest is elevated in patients with essential hypertension while cardiac output is normal ^(19, 37, 83). Early phases, e.g. borderline hypertension constitute exceptions as cardiac output may be increased as well ⁽³⁰⁾.

The underlying mechanism by which peripheral vascular resistance is elevated is not fully understood. A change of the wall/lumen ratio in the resistance vessels, e.g. due to smooth muscle hypertrophy ⁽⁸⁶⁾ or water logging due to increased sodium content of the vessel wall ⁽⁷³⁾ is conceivable.

Increased vasoconstrictor nerve activity has not been considered an important mechanism for maintenance of the elevated resistance and recent microneurographic studies have not revealed higher activity in efferent sympathetic nerves in hypertensive patients than in normals ⁽⁷⁸⁾.

Elevated levels of plasma catecholamines have recently been reported in essential hypertension ^(19, 4) which may seem surprising in view of the finding that urinary excretion of norepinephrine is not increased ⁶.

Another factor of importance for vascular resistance is blood viscosity. It has been reported that patients with essential hypertension have increased blood viscosity ⁷⁰⁾ but it should be pointed out that in vitro measurement of this parameter may be an unreliable indicator of in vivo viscosity ¹⁾. Others consider viscosity to be within normal limits in essential hypertension ⁽⁴⁷⁾.

In summary elevation of blood pressure in established essential hypertension is an effect of inappropriately elevated total peripheral resistance in relation to cardiac output.

Plasma renin activity

A humoral pressor substance of renal origin was first described – and given the name renin – by Tigerstedt and Bergman in 1898 ⁽⁷²⁾. The classic observation in 1934 by Goldblatt et al. that renal ischemia produced hypertension ⁽²²⁾ caused a renewal of the interest in renin. Later the renin-angiotensin system was described ^(4, 46, 80, 82, 74). Aldosterone was isolated ⁽⁸⁸⁾ and the relation between renin/angiotensin and aldosterone was discovered ^(23, 34, 43).

Initiated by these fundamental studies, a number of investigations have increased our knowledge of renin, angiotensin and aldosterone in various kinds of hypertension.

As a rule, plasma renin activity is not elevated in benign essential hypertension ^(18, 28, 78) and aldosterone secretion is normal ^(15, 36). Low plasma renin activity is seen in approximately 25 % of patients with benign essential hypertension ^(18, 28). On the other hand, elevated renin levels and increased excretion of aldosterone are prevalent findings in malignant hypertension ^(32, 34).

In 1960 Laragh suggested that the vascular damage in malignant hypertension was related to elevated levels of angiotensin ^(32, 35). Support for this hypothesis is given by results from animal research in which excess renin/

angiotensin has been shown to cause vascular lesions of the same appearance as those in malignant hypertension^(8, 21, 22, 30)

Recently it has been suggested that low plasma renin in patients with essential hypertension is associated with a reduced risk of development of cardiovascular complications such as stroke and myocardial infarction⁽⁵⁾. A positive relationship has been reported also between plasma renin and the severity of hypertensive retinopathy⁽⁷⁰⁾.

If these observations hold true it would seem to be preferable to employ therapy that not only lowers blood pressure but reduces renin as well in the care of hypertensive patients.

A number of antihypertensive drugs currently in use have been studied with regard to their effect on plasma renin activity. Elevation of plasma renin has been reported as a result of treatment with thiazide diuretics^(2, 44), hydralazine⁽³⁸⁾, diazoxide⁽³²⁾ and sodium nitroprusside⁽³¹⁾.

On the other hand, drugs with adrenergic inhibitory effects may cause reductions of plasma renin activity. This has been shown e.g. for alpha-methyldopa⁽⁴⁵⁾, clonidine⁽⁴⁶⁾, propranolol^(6, 40, 80) while there are different opinions regarding the effect of phentolamine^(40, 80).

In summary Plasma renin activity is usually elevated in malignant hypertension and normal or low in benign essential hypertension. The observation that low plasma renin may be associated with a reduced risk of cardiovascular complications deserves further investigation. Implications regarding the possibilities of a prophylactic effect of plasma renin lowering therapy are not justified at present.

Beta-adrenergic blockers in hypertension

Antihypertensive effect of beta-adrenergic blocking drugs was first reported with use of pronethalol^(48, 49) a drug that has not been

exploited in view of its tumor producing action in mice⁽⁴⁰⁾.

The first report of antihypertensive action of currently used beta-adrenergic blocking agents was published in 1964 by Prichard and Gillam⁽⁴⁰⁾. They reported effective reduction of blood pressure following oral administration of propranolol. Later they extended their studies to larger numbers of patients^(80, 81) and concluded that propranolol was an equally potent antihypertensive agent as e.g. guanethidine with the advantage of not producing postural or exercise hypotension⁽⁸¹⁾. Subsequently many positive reports have been published regarding the usefulness of propranolol in hypertension^(30, 25, 41) although occasionally no effect has been observed⁽²⁷⁾. Other beta-adrenergic blocking agents have also proved to be useful in hypertension, e.g. alprenolol⁽⁷¹⁾, oxprenolol⁽¹²⁾ and practolol⁽⁸²⁾.

From a theoretical point of view the antihypertensive action of propranolol may seem paradoxical considering its predominantly cardiac effects⁽³⁰⁾. Furthermore, as acute intravenous administration of propranolol does not reduce blood pressure in patients with normal rhythm⁽⁷⁶⁾ it has been difficult to understand the mode of action of the drug.

Several suggestions have been offered in an attempt to explain how blood pressure is reduced by propranolol. Reduction of cardiac output does not by itself lower blood pressure due to a compensatory increase of peripheral vascular resistance⁽⁷⁵⁾. Resetting of baroreceptors has been suggested in response to reduction of the cardiac component of transient rises of blood pressure^(50, 51).

However so far this hypothesis has not been confirmed.

In animal research it has been shown that reduction of blood pressure may result from the local anesthetic effect of propranolol causing adrenergic nerve blockade⁽¹³⁾. Hypotension could be produced in rabbits also by the denervation of propranolol which retains the

local anesthetic effect of l-propranolol but has less than one hundredth of the beta-adrenergic blocking effect ⁽¹⁾ However the anti-hypertensive effect of d-propranolol was negligible when given to 35 hypertensive patients whereas dl-propranolol proved to be effective ⁽⁷⁷⁾

The importance of this mechanism in man therefore remains doubtful.

Plasma volume contraction is not a reproducible finding during chronic propranolol therapy ^(56, 67) and can not be responsible for the reduction of arterial pressure

Finally adaptation of peripheral vascular

resistance in response to long-term reduction of cardiac output ⁽⁶⁸⁾ and relation of blood pressure reduction to suppression of plasma renin activity ⁽⁶⁾ have been suggested Both possibilities will be commented upon in the discussion as part of the present study was devoted to these problems.

In summary: Beta-adrenergic blockade has been shown to reduce blood pressure effectively in hypertensive patients without causing postural hypotension or reduction of blood pressure during exercise. There are controversial opinions regarding its mode of action and this will be discussed further in this paper

angiotensin has been shown to cause vascular lesions of the same appearance as those in malignant hypertension^(8, 21, 22, 30)

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However so far this hypothesis has not been confirmed

In animal research it has been shown that reduction of blood pressure may result from the analgesic effect of propranolol causing adrenergic nerve blockade⁽¹³⁾. Hypotension could be produced in rabbits also by the intravenous infusion of propranolol which retains the

as the study was started with a four week placebo period. To further reduce risks during the placebo period all patients were instructed to contact the clinic should diastolic home blood pressure rise above 120 mm Hg at two successive measurements.

After being informed about all details of the study except the initial placebo period, both verbally and in writing, all patients gave their written consent to participate.

Material

Initially 19 patients were recruited to the study. They had all been investigated for their hypertension at either the University of Michigan Medical Center or the Veterans Administration Hospital in Ann Arbor. There were two early drop-outs. One patient showed a rise of diastolic home blood pressure to over 140 mm Hg already during the first week of placebo treatment and had to resume active therapy. The second patient was normotensive (less than 150/90 mm Hg) at the end of the placebo

period. In addition, his home blood pressure had been normal throughout the four weeks of placebo treatment.

Of the remaining 17 patients two refused the second hemodynamic study at the end of four weeks of propranolol therapy thereby reducing the number of patients who completed the study to 15. No patient was withdrawn during the study e.g. due to side-effects or unsuccessful catheterizations.

The diagnosis of essential hypertension was made after careful clinical evaluation (history and physical examination) laboratory studies including serum electrolytes, serum creatinine, urinary excretion of aldosterone, catecholamines or vanillomandelic acid, tests for proteinuria, urine sediment and rapid sequence intravenous pyelograms.

The average age was 45 years (range 26-66) and the average known duration of hypertension was 7 years (range 0-17). All patients had benign hypertension as judged by fundoscopic examination. Only one patient had left ventricular hypertrophy as estimated by chest

Table I Patient information.

Patient	Age years	Race*	Duration of hyper- tension years	EKG**	Serum creati- nine mg/100 ml	Creati- nine clear- ance 1/24 h	LVH*** EKG/ chest X-ray	Pre- vious therapy	Initial § Blood Pressure mm Hg	Placebo ¶ Blood Pressure mm Hg
1 F.L.	50	C	10	I	0.9	180	-	A D	130/90	159/98
2 H.T.	50	B	2	II	1.1	216	-	D	142/112	150/110
3 L.W.	55	B	2	I	1.0	142	-	D	144/100	155/110
4 L.A.	53	C	7	I	1.0	125	-	D	125/78	180/120
5 T.D.	66	C	13	I	1.2	88	-	II	194/97	169/86
6 D.B.	26	C	0	I	1.1	N.A.	-	-	140/91	163/95
7 R.R.	40	C	17	II	1.6	162	-	G D	162/98	174/137
8 M.E.	27	B	8	0	1.1	177	-	D	121/92	134/95
9 M.D.	52	C	0	I	0.9	149	-	-	136/99	155/108
10 L.K.	49	C	7	II	1.1	N.A.	+	A D	182/113	200/128
11 J.K.	45	C	7	II	1.2	N.A.	-	A D	149/107	161/111
12 M.P.	44	C	9	I	1.0	155	-	-	152/96	177/107
13 G.H.	41	C	10	II	1.1	191	++	A D	179/123	184/105
14 A.P.	43	C	0	II	1.0	150	-	D	143/98	140/96
15 F.P.	35	C	5	II	0.9	198	-	-	164/130	162/110

*C Caucasian B Black **Keith Wagener and Barker (1939) classification. ***Left ventricular hypertrophy §A Alpha-methyldopa, D Diuretics G Guanadrel and R Reserpine ¶ At admission to study measured in the recumbent position ¶¶ After 4 weeks of placebo ¶¶ A Not available

X-ray and EKG while one patient fulfilled the EKG criteria only. One patient had impaired renal function as judged by serum creatinine (1.6 mg/100 ml) while the remainder had normal serum creatinine concentration.

Before admission to the study 11 patients were receiving antihypertensive therapy regularly. This consisted of diuretics in 5, alpha-methyldopa and diuretics in 4, reserpine in 1 and guanadrel and diuretics in 1. The remaining four patients were untreated although 3 of them had been treated earlier. For information on individual patients see Table I.

Methods

Clinic blood pressures

All out-patient visits were in a special research clinic, the Upjohn Center for Clinical Pharmacology at the University of Michigan Medical Center, Ann Arbor. Throughout the study the same two nurses recorded all blood pressures. Their correlation was checked frequently by simultaneous readings using a "Y" connection. A mercury manometer attached to a 14 cm wide cuff was used.

Readings were always made in the morning in a quiet air-conditioned room. Recumbent pressures were measured after 15 minutes of rest and standing pressures were recorded 2 minutes after assumption of upright posture.

Recumbent and standing pulse rates were measured accordingly. Phase 4 (disappearance) of Korotkoff sounds was taken as the diastolic end point. With few exceptions the average of 3 readings was calculated.

Clinic blood pressure was recorded at the beginning of the study, after 4 weeks of placebo treatment and after 2 and 4 weeks of oral propranolol therapy.

Home blood pressures

All patients were carefully instructed how to measure blood pressure and their accuracy

was checked by the nurses using a "Y" connection. They were then provided with specially designed home blood pressure sphygmomanometers (Propper) and instructed to record home blood pressure mornings and evenings in the recumbent and standing positions. Recumbent pulse rate was also recorded. Daily measurements of home blood pressure and pulse rate were continued throughout the study. Special sheets were used for recording of these parameters and side-effects or comments. Home blood pressures were consistently lower than corresponding recordings in the clinic. Patient No. 5 was an exception in this respect as his recordings at home were higher than in the clinic. Clinic blood pressures were significantly correlated to home blood pressure both after 4 weeks of placebo (Fig. 1) and after 4 weeks of propranolol (Fig. 2) independently of whether morning or evening home blood pressures were used.

Hospital investigations

After 4 weeks of placebo treatment all patients were hospitalized during 4 days in a metabolic research ward - the Clinical Research Unit - for baseline laboratory studies. Placebo treatment was continued during the stay in hospital. Studies of 12-hour urinary excretion of catecholamines were made twice with collections starting at 8 p.m. A 24-hour endogenous creatinine clearance was made and, ending on the morning of the fourth day, studies of 24-hour urinary excretion of aldosterone, sodium and potassium.

A simple no-added-salt diet was introduced and the patients were instructed repeatedly by a dietician regarding the continuation of a similar diet following discharge.

An extensive number of blood (serum) and urine tests were made repeatedly during the study. EKG and chest x-ray were performed initially and following the placebo and propranolol periods.

Hemodynamic studies

Cardiac output determinations

The hemodynamic studies were performed twice in every patient. The initial study was made after 4 weeks of placebo treatment and the second was performed after the propranolol period. The hemodynamic studies were made in the morning with the patients fasting.

An 18-gauge teflon catheter was inserted into the left brachial artery and a polyethylene catheter (PE 50) was introduced percutaneously into the left antecubital vein and propagated to a central vein. Systemic arterial pressure was measured by a Statham strain gauge transducer (P 23 G) kept at a level 5 cm below the sternum.

Cardiac output was determined by the indicator dilution technique⁽⁴²⁾ using indocyanine green (Cardio-Green) and a Gilford densitometer. Direct dye calibration was performed after each procedure by the addition of 5, 10 and 15 μ l of Cardio-Green (3 mg/ml) to 10 ml aliquots of blood which were then drawn through the densitometer.

Respiration was monitored continuously by means of a bellows attached around the chest and connected to a strain gauge by a rubber tube.

Standard lead I of the EKG together with intra-arterial blood pressure, respiration and dye curves were recorded on a Gilson poly graph.

Stroke volume (SV) was calculated by dividing cardiac output (Q) by heart rate (HR). Total peripheral resistance (TPR) was obtained by dividing mean arterial pressure (MAP) by Q and was expressed in arbitrary units (u).

Body surface area (BSA) was calculated from the formula, $BSA = W^{0.425} \times H^{0.725} \times 71.84$ (DuBois and DuBois) where W = weight in kg and H = height in cm.

By compensating for BSA the corresponding indices QI, TPRi and SVi were calculated

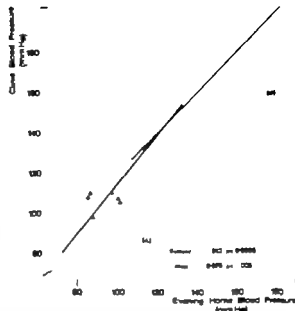


Fig. 1. Correlations between recumbent clinic blood pressure at the end of 4 weeks of placebo treatment and average recumbent evening home blood pressure during the last week of placebo. Readings in patient No. 5 are within brackets and have not been included in the calculations (see text).

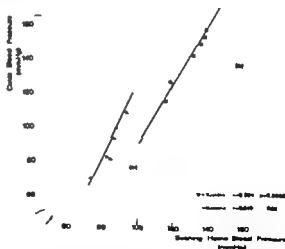


Fig. 2. Correlations between recumbent clinic blood pressure at the end of 4 weeks of propranolol treatment and average recumbent evening home blood pressure during the last week of propranolol. Readings in patient No. 5 are within brackets and have not been included in the calculations (see text).

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additional dose of propranolol, 0.05 mg/kg, bringing the total dosage to 0.10 mg/kg. Cardiac output was again determined after 10 min. Finally a further doubling of the dosage was made by giving a third injection of propranolol. To compensate for wear-off effects a 10% addition of dosage was made at this time bringing the cumulated dose to 0.22 mg/kg. Again hemodynamic measurements were repeated after 10 min.

A second 10-min period of 45° head-up tilt was then started at the end of which cardiac output measurements were repeated. Finally with the patients tilted back to horizontal, isoproterenol, 3 µg/min, was again infused intravenously during 3 min with recording of heart rate.

Hemodynamic study II

The second hemodynamic study was made at the same time of day and under similar conditions as the first study but this time following 4 weeks of oral propranolol therapy. Cardiac output during rest and after 10 min of tilt was determined as before. Furthermore, the baroreceptor reflex was re-assessed. Finally the infusion of isoproterenol, 3 µg/min, over 3 min was repeated.

Nitroglycerine test

In order to evaluate the possibilities of estimating the degree of beta-adrenergic blockade by a non-invasive test nitroglycerine was given sublingually and the patients assumed upright posture while their pulse rate was recorded. This was done 4 times during the study.

The nitroglycerine test was originally proposed by Fitzgerald as a simple means of estimating the degree of beta-adrenergic blockade⁽¹⁷⁾ and he has also shown that the nitroglycerine test is more specific than e.g. Valsalva's manoeuvre, hyperventilation, sustained hand grip assumption of upright posture, mild exercise and maximal exercise because the effect of atropinization is less pronounced⁽¹⁸⁾

The nitroglycerine test as employed in the present study differed from Fitzgerald's original description in that the patients were told to stand up after the administration of nitroglycerine. This was made to ensure optimal stimulation of heart rate.

After recording heart rate during rest the patients were given a nitroglycerine tablet, 0.4 mg, sublingually and at the same time they assumed upright posture. Pulse rate was then recorded continuously during 6 min and the highest pulse rate during any 30-second period was compared to the initial heart rate.

The test was performed in the same manner after four weeks of placebo treatment, after intravenous injection of propranolol, 0.22 mg/kg, (approximately 25 min after the injection was given) after two weeks of oral propranolol therapy at 160 mg daily (approximately 1 hour after the last dose) and finally after four weeks of oral propranolol therapy at 160 or 320 mg daily.

The change of heart rate due to this manoeuvre was then compared to the change of heart rate due to isoproterenol infusion. It was also correlated to plasma propranolol concentration.

Plasma propranolol concentration

Plasma propranolol concentrations were determined by fluorimetric technique⁽¹⁹⁾ by Ayerst Laboratories, Montreal, Canada through courtesy of Dr Henry L. LeMien Jr.

Blood samples were drawn approximately 20 min after the completion of the intravenous administration of propranolol 0.22 mg/kg and again following two and four weeks of oral propranolol therapy approximately 1-2 hours after the last dose.

The plasma was separated by centrifugation and chilled to 4° C before being sent in an isolated tube for analysis.

Plasma propranolol concentration was related to the change of blood pressure and other hemodynamic parameters as well as to changes

of plasma renin activity and to changes of heart rate due to sublingual nitroglycerine and intravenous isoproterenol. In view of recent observations⁽⁶⁰⁾ the logarithm of plasma propranolol concentration was also used for correlations.

Plasma renin studies

Analysis of plasma renin activity (PRA) was made after 4 weeks of placebo treatment in the recumbent position and again after 10 min of 45° head-up tilt. Furthermore, PRA was analysed after acute intravenous administration of propranolol 0.22 mg/kg and repeated tilt for 10 min. Finally PRA was determined following 4 weeks of oral propranolol therapy (160–320 mg daily) again after 10 min of tilt. Studies were not made in patient No. 6.

For each sample 10 ml of arterial blood was collected in a prechilled tube containing approximately 10 mg of EDTA Na. The tube was immediately placed in ice and centrifuged within 20 min in a refrigerated centrifuge to recover the plasma. The plasma samples were then frozen and stored until all samples were collected.

For analysis a radioimmunoassay method was used^(34, 61) by which PRA is determined by measurement of the generated angiotensin I*. Duplicate analyses were made of all specimens and the results were compared to a standard curve obtained from standard solutions of angiotensin I. Nonspecific activity was determined in specimen No. 1 and subtracted from the results of analysis of samples No. 1–3 (which were collected on the same day). Nonspecific activity was also determined in specimen No. 4 and subtracted.

The error of duplicate analyses varied between 4.2 and 7.9 % ($\sqrt{\frac{d^2}{n}}$ expressed in percent of the mean, d = the difference between duplicate analyses)

The change of stimulated (tilted) PRA after 4 weeks of oral propranolol therapy has been correlated to changes of blood pressure and other hemodynamic parameters.

Side effects

At each clinic visit patients were asked to report any adverse effects or complaints. They were then asked 17 questions from a standardized questionnaire relating to cardiopulmonary gastrointestinal, neurological and miscellaneous symptoms. A brief physical examination was also made at each visit directed at ruling out cardiac insufficiency and bronchial obstruction. Weight was recorded at each visit.

Statistical methods

Statistical methods included Student's *t*-test for group differences and paired data analysis, Wilcoxon's test and standard methods for calculation of correlation and regression coefficients (Documents Geigy 1962). Most analyses were made using the computer program Midas (Constat) of the Statistical Research Laboratory of the University of Michigan. Probabilities less than 0.05 were considered significant.

Critical aspects of the material and methods

A. Effect of previous treatment

Eleven of the 15 patients were being treated with antihypertensive drugs before entering the study. This therapy usually consisted of hydrochlorothiazide or alpha-methyldopa. In spite of the 4-week placebo period it is conceivable that some influence on blood pressure may have remained at the start of propranolol treatment although the effects should have been minimal and would tend to reduce the antihypertensive action of propranolol.

The effects on plasma renin activity are more interesting since both thiazides and alpha-methyldopa affect PRA. However it has been reported that the increase of PRA during chronic thiazide therapy is temporary and that PRA falls towards the initial level during treatment⁽³⁾ Furthermore, it has been shown that elevated PRA falls to a normal level already during the first week after discontinuance of thiazide therapy⁽¹⁰⁾ The effect of alpha-methyldopa on PRA is also relatively brief as illustrated by the finding that PRA had increased to the initial level 2 weeks after withdrawal of therapy⁽⁴¹⁾ It can be concluded therefore that the effects of previous therapy were insignificant.

B Use of placebo treatment

It can be argued from an ethical point of view that, with our present knowledge of hypertension, placebo should not be given. In the present study attempts were made to minimize the effects of withdrawal of active therapy by selecting patients with relatively mild hypertension and by limiting the placebo period to 4 weeks. Furthermore, an extra precaution was taken by providing all patients with equipment for measurement of home blood pressure and instructing them to call if diastolic blood pressures over 120 mm Hg were recorded repeatedly

C. Effect of age

The age distribution in the present study (26-66 years, mean 45 median 46) was fairly wide

However age was evenly distributed. It has been shown that older subjects have lower resting cardiac output and higher total peripheral resistance⁽³⁷⁾ It is also known that age reduces the hemodynamic effects of propranolol⁽⁷⁾ For these reasons, age has been correlated to changes of blood pressure and other hemodynamic alterations as well as to changes of plasma renin activity In no instance have significant correlations been observed between age and alterations of hemodynamic parameters or PRA This indicates that age did not affect the results of the present study Finally as comparisons are made on a within-patient basis and few attempts are made to compare groups the importance of age is further reduced

D Effects of blood loss

As blood was not reinfused during the hemodynamic studies and as a number of blood tests and sampling of blood for PRA was made during the study it is conceivable that a reduction of hemoglobin concentration or hematocrit might occur which could affect blood viscosity and thereby limit the possibilities of comparing e.g. TPR before and after oral propranolol therapy For this reason hemoglobin concentration and hematocrit before the first and after the second hemodynamic study were compared statistically (paired t test) No significant changes were seen of either parameter indicating that significant changes of blood viscosity had not occurred.

RESULTS

Effect on blood pressure

Significant reductions of blood pressure were seen both with regard to measurements made in the clinic (Table II) and at home (Table III). A significant reduction of home blood pressure was seen already during the first week of propranolol therapy but there was an insignificant further fall of blood pressure also during the following weeks. Individual changes of clinic blood pressure are given in Fig. 3 and 4.

Pulse rate was also significantly reduced both in the recumbent and standing position. (Table II)

The changes of systolic blood pressure (recumbent and standing) as measured in the clinic after 4 weeks of oral therapy were significantly correlated to plasma propranolol con-

centration ($r = 0.782$, $p < 0.005$ and $r = 0.541$, $p < 0.05$). The changes of diastolic blood pressure recumbent or standing were not significantly correlated to plasma propranolol concentration, ($r = 0.358$, n.s. and $r = 0.283$, n.s.). Furthermore, the changes of both systolic and diastolic blood pressure, recumbent and standing, between the second and fourth week of propranolol therapy were significantly correlated to plasma propranolol concentration after 4 weeks of treatment (correlation coefficients between 0.525 and 0.699, $p < 0.05$ to $p < 0.01$).

Blood pressure changes in the 8 patients treated with 160 mg daily for 4 weeks were similar to those of the group in which dosage was increased to 320 mg. However a tendency towards continuing reduction of systolic blood pressure was observed in the latter group (Fig. 5).

Table II. Change of clinic blood pressure and heart rate.

	Recumbent			Standing		
	Heart rate	BP syst.	BP diast.	Heart rate	BP syst.	BP diast.
Initial*	77.9	150.8	102.3	88.4	148.4	106.8
Placebo**	82.9	164.3	107.7	92.3	158.1	111.2
Difference	5.0	13.5	5.4	4.1	9.7	4.4
Significance p <	n.s.	0.05	n.s.	n.s.	n.s.	n.s.
After 2 weeks of oral Propranolol						
Difference***	67.8	136.7	91	74.3	137.3	97.7
Significance p <	15.1	27.6	16.6	18.2	20.8	13.5
	0.0001	0.0001	0.0005	0.0001	0.001	0.005
After 4 weeks of oral Propranolol						
Difference***	64.6	135.7	87.8	68.8	135.4	95.9
Significance p <	18.3	28.6	19.9	23.7	22.7	15.2
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0005

*At admission to study **Following 4 weeks of placebo treatment

***In comparison to Placebo. Statistical comparison with paired data t-test

Table III Change of recumbent home blood pressure and heart rate

	Morning			Evening		
	Heart rate	BP syst.	BP diast.	Heart rate	BP syst.	BP diast.
Week 4 Placebo	74.0	147.5	101.6	79.6	148.1	98.8
Week 1 Propranolol	69.8	134.0	88.7	72.5	132.3	89.0
Difference	4.2	13.5	12.9	7.1	15.8	9.8
Significance $p <$	0.01	0.001	0.0001	0.001	0.0005	0.005
Week 2 Propranolol	68.7	132.9	86.6	72.4	132.4	86.8
Difference	5.3	14.6	15.0	7.2	15.7	12.0
Significance $p <$	0.005	0.005	0.0005	0.005	0.0001	0.0005
Week 3 Propranolol	69.7	131.3	85.1	72.7	127.3	84.9
Difference*	4.3	16.2	16.5	6.9	20.8	13.9
Significance $p <$	0.01	0.001	0.0001	0.005	0.0001	0.0005
Week 4 Propranolol	67.0	133.1	86.7	70.5	129.9	83.9
Difference	7.0	14.4	14.9	9.1	18.2	14.9
Significance $p <$	0.005	0.001	0.0001	0.005	0.0001	0.0001

*In comparison to week 4 of placebo treatment. The average of seven recordings per week are given.
Statistical comparison with paired data t-test.

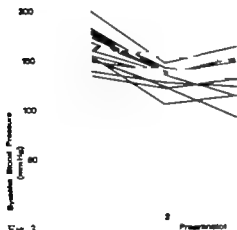


Fig 3

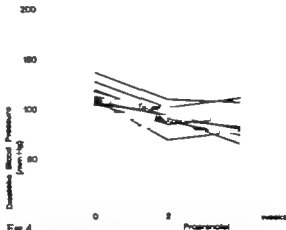


Fig 4

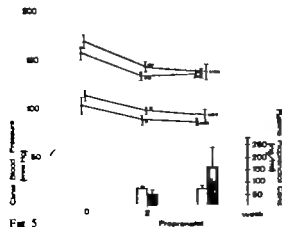


Fig 5

Fig 3
Individual changes of recumbent systolic blood pressure measured in the clinic (total recordings were after 4 weeks of placebo treatment. Propranolol dosage was 160 mg daily during weeks 1-2 and 160 or 320 mg daily (av. 250) during weeks 3-4.

Fig 4
Individual changes of recumbent diastolic blood pressure measured in the clinic. Therapy as in Fig 3.

Fig 5
Clinic blood pressure and plasma propranolol concentration in patients receiving propranolol 160 mg daily for 4 weeks (circles and unfilled bars) and in patients receiving 160 mg daily for 2 weeks and 320 mg daily for 2 weeks (triangles and striped bars). Mean values \pm SEM. Statistical comparisons are with the groups (paired t-test). Probabilities as in Fig 3.

Hemodynamic effects

Resting data during placebo

During placebo treatment the following hemodynamic parameters (\pm S.E.M.) were recorded (Table IV)

- 1) Q_i 2.76 ± 0.15 l/min m^2
- 2) MAP 108.3 ± 2.5 mm Hg.
- 3) TPR 41.3 ± 2.7 u/ m^2
- 4) HR 76.0 ± 2.3 beats/min.
- 5) SVI 37.3 ± 2.6 ml/ m^2

Effects of acute intravenous propranolol

Intravenous administration of propranolol, 0.05 mg/kg, resulted in (Fig. 6 and 7)

- 1) Q_i fell from 2.76 to 2.29 l/min m^2 ($p < 0.01$)
- 2) MAP did not change significantly at 107.2 mm Hg.
- 3) TPR increased from 41.3 to 48.5 u/ m^2 ($p < 0.05$)
- 4) HR fell from 76.0 to 71.8 beats/min ($p < 0.005$)
- 5) SVI did not change significantly at 32.4 ml/ m^2

After doubling the dose of propranolol to a total cumulated dosage of 0.10 mg/kg the

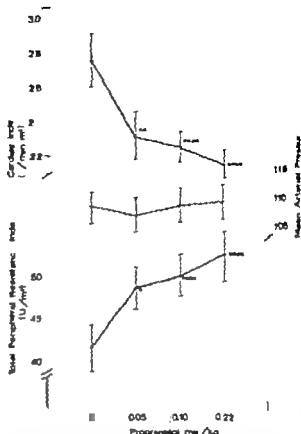


Fig. 6 Effects of increasing doses of propranolol i.v. on cardiac index, mean arterial pressure (dashed lines) and total peripheral resistance index. The cumulated doses indicated on the x-axis were given 10 min apart. Mean values \pm S.E.M. are given. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$ **** $p < 0.001$ (paired t-test) Comparisons to initial values

Table IV Hemodynamic changes at rest.

	Placebo	Propranolol 0.22 mg/kg i.v.	Difference	Significance since $p <$	Propranolol orally ^a	Difference ^{ab}	Significance $p <$
Cardiac index l/min m^2	2.76	2.18	0.58	0.001	2.35	0.41	0.01
Mean arterial pressure mm Hg	108.3	109.9	1.7	n.s.	102.1	6.2	0.05
Total peripheral resistance index u/ m^2	41.3	52.3	11.0	0.005	43.9	2.6	n.s.
Heart rate beats/min	76.0	67.1	8.9	0.0001	59.6	16.4	0.0001
Stroke volume and ml/ m^2	37.3	33.0	4.3	n.s.	40.2	2.9	n.s.

Four weeks of oral treatment 160–170 mg daily ^{ab}Compared to measurement after placebo

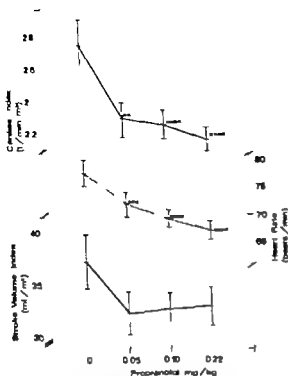


Fig 7
Effects of increasing doses of propranolol IV on cardiac index, heart rate (dashed lines) and stroke volume index. Dose and statistics as in Fig 6

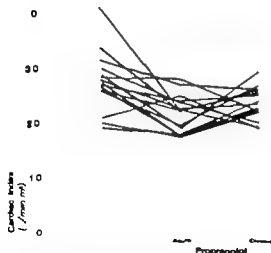


Fig 8
Individual changes of resting cardiac index. Initial measurements are after 4 weeks of placebo treatment. Acute propranolol indicates measurement after IV administration of 0.22 mg/kg. Chronic propranolol indicates 4 weeks of oral treatment 160-320 mg daily

hemodynamic results were (comparisons to the corresponding data during placebo)

- 1) Q_i fell to 2.25 l/min m^2 ($p < 0.001$)
- 2) MAP did not change significantly at 109.1 mm Hg.
- 3) TPR_i increased to 49.9 u/m^2 ($p < 0.001$)
- 4) HR fell to 69.4 beats/min ($p < 0.0001$)
- 5) SVI did not change significantly at 32.7 ml/m^2 (Fig. 6 and 7)

By further increasing the dosage of propranolol to a cumulated dosage of 0.22 mg/kg the hemodynamic results were (comparisons to the corresponding data during placebo)

- 1) Q_i fell to 2.18 l/min m^2 ($p < 0.001$)
- 2) MAP did not change significantly at 109.9 mm Hg.
- 3) TPR_i increased to 52.3 u/m^2 ($p < 0.001$)
- 4) HR fell to 67.1 beats/min ($p < 0.001$)
- 5) SVI did not change significantly at 33.0 ml/m^2 (Fig. 6 and 7)

Effects of prolonged oral propranolol

Four weeks of oral propranolol therapy resulted in (Table IV and Fig. 9)

- 1) A remaining significant reduction of Q_i (from 2.76 to 2.35 l/min m^2 $p < 0.01$)
- 2) A significant reduction of MAP from 108.3 to 102.1 mm Hg ($p < 0.05$)
- 3) A re-adjustment of TPR_i to a level not significantly higher than during placebo treatment (43.9 u/m^2)
- 4) A significant reduction of HR to 59.6 beats/min ($p < 0.0001$) This was also significantly lower than HR after acute IV propranolol
- 5) A non-significant increase of SVI to 40.2 ml/m^2

Individual changes of cardiac index are illustrated in Fig. 10.

Analysis of the hemodynamic changes in individual patients revealed that while 12 patients showed a decrease of MAP following prolonged oral propranolol therapy 3 patients

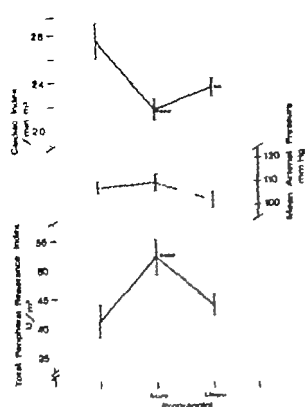


Fig 9
Effects of acute IV (0.22 mg/kg) and 4 weeks of oral propranolol (160-320 mg daily) on cardiac index, mean arterial pressure (dashed lines) and total peripheral resistance index. Statistics as in Fig. 6

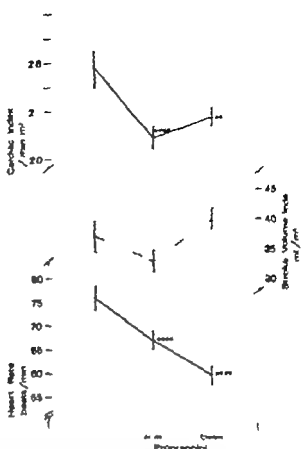


Fig 10
Effects of acute IV (0.22 mg/kg) and 4 weeks of oral propranolol (160-320 mg daily) on cardiac index, stroke volume index (dashed lines) and heart rate. Statistics as in Fig. 6

Table V Hemodynamic changes during tilt.

	Placebo (n = 15)				Propranolol IV (n = 12)				Propranolol orally** (n = 12)			
	Rest	Tilt	Difference	Significance p<	Rest	Tilt	Difference	Significance p<	Rest	Tilt	Difference	Significance p<
Cardiac index l/min m ²	2.76	2.19	0.57	0.005	2.19	1.60	0.59	0.0005	2.44	2.04	0.40	0.005
Mean arterial pressure mm Hg	108.3	119.6	11.3	0.0005	106.4	98.3	8.1	n.s.	103.4	110.3	6.9	0.005
Total peripheral resistance index U/m ²	41.3	56.5	15.2	0.0001	50.4	63.8	13.4	0.005	42.6	54.6	12.0	0.0005
Heart rate beats/min	76.0	84.9	8.9	0.005	69.5	69.2	0.3	n.s.	57.7	60.6	2.9	0.01
Stroke volume index ml/m ²	37.3	26.4	10.9	0.0001	32.0	22.9	9.1	0.0001	42.7	34.4	8.3	0.0005

*0.22 mg/kg IV **oral treatment for 4 weeks, 160-320 mg daily

(No 10, 11 and 12) showed no decrease or an increase. By comparing the two groups it was shown that there was no significant difference of QI and TPRi during the placebo period. Neither was QI significantly different after oral propranolol treatment. However the difference of MAP was reflected by a significant difference of TPRi after oral propranolol therapy. MAP was also significantly different between the two groups already during acute beta-adrenergic blockade (105.9 vs 122.2 mm Hg, $p < 0.05$) (Fig. 11).

None of the hemodynamic changes (except for the previously mentioned reductions of clinic blood pressure) were significantly correlated to plasma propranolol concentration after 4 weeks of oral therapy.

Effects of tilt

A After placebo

There were no distressing symptoms during the initial 10 min tilt. As expected, there were reductions of QI and SVI while HR and TPRi rose significantly resulting in a rise of MAP (Table V).

B After IV propranolol

Tilt after intravenous administration of propranolol caused disturbing postural symptoms in several patients and one patient actually fainted just after the hemodynamic measurements were made. In comparison to the hemodynamic parameters in the recumbent position after I V propranolol significant reductions of SVI and QI occurred while TPRi increased. The net effect on MAP was a non-significant reduction (Table V).

C After oral propranolol

There were no complaints of discomfort during tilt after 4 weeks of propranolol therapy and no patient appeared to be close to fainting. Again significant reductions of SVI and QI

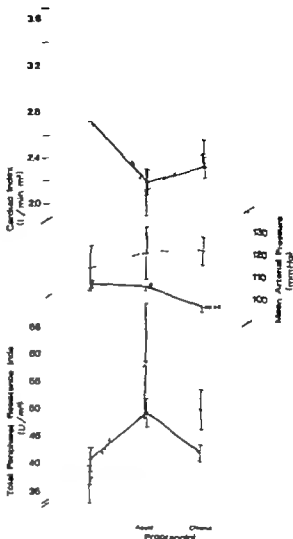


Fig. 11

Hemodynamic differences between 12 patients with reduced intra-arterial pressure and 3 with unchanged or increased MAP (dashed lines). Mean values \pm SEM are given. Probabilities as in Fig. 6 (Student's *t*-test). Comparisons between groups.

and a significant increase of TPRi were seen. As in the placebo situation, there was an increase of MAP and a small but significant increase of HR (Table V).

Effect on baroreceptor sensitivity

Only in 7 patients were statistically significant correlations between intra-arterial systolic

blood pressure and R-R interval (BP/R-R slope) obtained following IV angiotensin both after placebo and after 4 weeks of oral propranolol therapy. The average initial regression coefficient was 4.84. After treatment the average regression coefficient was 7.64. The difference, 2.80 did not quite achieve statistical significance ($p = 0.115$) (paired t -test).

The change of the BP/R-R slope was not significantly correlated to plasma propranolol concentration.

Effect of Isoproterenol Infusions

In the untreated state, recumbent resting heart rate increased from 73.1 to 85.2 beats/min ($p < 0.0005$) in response to intravenous infusion of isoproterenol 1 μ g/min during 3 min. By increasing the rate of infusion to 2 μ g/min for 3 min heart rate increased to 100.7 beats/min ($p < 0.0005$). Finally following 3 min of isoproterenol infused at a rate of 3 μ g/min heart rate accelerated to 112.8 beats/min bringing the total increase to 39.8 beats/min ($p < 0.0005$).

Intravenous infusion of isoproterenol at the rate of 3 μ g/min for 3 min was repeated after intravenous administration of propranolol, 0.22 mg/kg. In this situation average heart rate only increased by 0.9 beats/min (n.s.) indicating a marked degree of beta-adrenergic blockade.

Finally after 4 weeks of oral propranolol therapy isoproterenol was again infused at a rate of 3 μ g/min over 3 min. The average increase of heart rate was only 5.3 beats/min ($p < 0.05$) again indicating marked beta-adrenergic blockade.

Effect on plasma renin activity

All patients were within the normal or low normal range in regard to resting plasma renin activity (PRA) after placebo. In response to 10 min of 45° head-up tilt average PRA rose from 151.7 to 248.7 ng/100 ml h (n.s.). Renew

Table VI Plasma renin activity (PRA) and sodium excretion.

Patient	PRA rest placebo	Urinary Sodium mEq/ 24 h	PRA tilt placebo	PRA tilt prop. IV **	PRA tilt prop. orally ***
1	40.3	160	59.4	27.6	14.0
2	228.2	67	434.0	318.0	89.2
3	54.4	105	149.0	68.9	12.4
4	443.4	55	709.3	722.9	20.4
5	48.8	46	52.7	N.A.	10.7
7	461.3	171	875.6	739.6	93.4
8	45.4	158	68.5	32.6	38.1
9	113.3	94	135.9	60.9	16.5
10	191.9	N.A.	146.4	93.0	6.9
11	125.4	N.A.	151.4	76.2	39.7
12	51.7	58	82.4	49.7	7.9
13	76.5	101	148.4	142.5	90.1
14	704.7	68	406.5	304.7	104.4
15	37.9	93	64.2	24.9	1.7
Average	151.7	98.0	248.7	190.0	39.0
S.E.M.	± 38.2	± 12.6	± 69.8	± 66.5	± 10.1

Significance _____ n.s. _____
 _____ n.s. _____
 _____ $p < 0.005$ _____
 _____ $p < 0.005$ _____

*ng/100 ml h

Propranolol 0.22 mg/kg IV *Propranolol orally for four weeks, 160-320 mg daily N.A. = Not available.

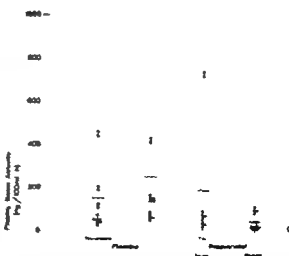


Fig. 12
 Plasma renin activity during placebo (resting recumbent and after 10 min 45° tilt) after propranolol 0.22 mg/kg IV (and repeated tilt) and finally following 4 weeks of oral propranolol therapy (and repeated tilt). Horizontal lines indicate mean values.

ed tilt after acute intravenous administration of propranolol 0.22 mg/kg caused a smaller increase of PRA to 190.0 ng/100 ml h (n.s.)

Finally after 4 weeks of oral propranolol therapy average PRA following 10 min of 45° tilt was only 39.0 ng/100 ml h. This is significantly lower than both the initial recumbent level ($p < 0.005$) and the level after initial tilt ($p < 0.005$)

Thus, the average stimulated (tilted) PRA following 4 weeks of oral propranolol was only 15% of the corresponding PRA during placebo. Individual PRA after prolonged propranolol therapy were all in the low or very low range (Fig. 12 and Table VI)

Change of PRA in relation to hemodynamic alterations

The change of tilted PRA from placebo to the level after oral propranolol treatment (average 209.3 ng/100 ml h) was not significantly correlated to either the reduction of recumbent systolic blood pressure as measured in the clinic ($r = 0.230$ n.s.) or to the change of diastolic recumbent blood pressure ($r = 0.449$ n.s.)

Nor was the change of tilted PRA significantly correlated to the changes of intra-arterial MAP, Q_1 or TPRI either in the recumbent or tilted position, (correlation coefficients 0.077–0.104 n.s.)

Table VII Correlations between decrease of home blood pressure (systolic and diastolic blood pressure measured in the recumbent and standing position mornings and evenings) and various initial parameters.

	Correlation coefficients		Probabilities	Remarks
Age	0.068	0.336	> 0.25	
Weight	0.058	0.442	> 0.25 < 0.20	
Heart Rate	0.046	0.663	> 0.25 < 0.02	1 of 8 correlations significant
Cardiac output	0.013	0.419	> 0.25 < 0.15	
Cardiac index	0.018	0.340	> 0.25	
Total peripheral resistance	0.154	0.700	> 0.25 < 0.02	2 of 8 correlations significant
Response to beta-adrenergic stimulation	0.314	0.589	> 0.25 < 0.05	1 of 8 correlations significant
Norepinephrine ^{***} excretion in urine	0.021	0.370	> 0.25 < 0.20	
Epinephrine ^{***} excretion in urine	0.007	0.395	> 0.25 < 0.20	
Total catecholamine ^{***} excretion in urine	0.013	0.419	> 0.25 < 0.15	
Aldosterone ^{***} excretion in urine	0.211	0.591	> 0.25 < 0.05	1 of 8 correlations significant
Creatinine clearance	0.014	0.408	> 0.25 < 0.20	

*I V infusion of isoprotrenol. **Two 12 h urine collections. ***One 24 h urine collection.

Furthermore, the change of tilted PRA, expressed in percent, was not significantly correlated to the percentage changes of MAP QI or TPRI either recumbent or tilted, (correlation coefficients 0.010-0.387 n.s.)

The individual responses were very similar particularly in regard to the effect of 4 weeks of oral propranolol. For example, the 3 patients showing unchanged or elevated intra-arterial pressure and significantly higher TPRI after oral propranolol did not show a different pattern in regard to PRA as their reductions of tilted PRA were 81, 90 and 96 % respectively as compared to the average of 85 %.

Finally the changes of PRA were not significantly correlated to plasma propranolol con-

centrations nor was the final level of PRA correlated to plasma propranolol concentration after 4 weeks of oral propranolol therapy.

Observations on predictability

As one of the aims of the present study was to try to identify factors that might help to predict the antihypertensive effect of propranolol a number of initial parameters were correlated to the change of blood pressure during oral propranolol therapy. Both clinic and home blood pressures, (systolic and diastolic, recumbent and standing, mornings and evenings) were used for analysis. Thus, the change of blood pressure

Table VIII Correlations between decrease of clinic blood pressure (systolic and diastolic blood pressure measured in the recumbent and standing position) and various initial parameters.

	Correlation coefficients		Probabilities		Remarks
Age	0.074	0.489	> 0.25	< 0.15	
Weight	0.062	0.491	> 0.25	< 0.15	
Heart rate	0.003	0.440	> 0.25		
Cardiac output	0.006	0.267	> 0.25		
Cardiac index	0.047	0.246	> 0.25		
Total peripheral resistance	0.058	0.229	> 0.25		
Response to beta-adrenergic stimulation*	0.102	0.341	> 0.25		
Norepinephrine** excretion in urine	0.015	0.389	> 0.25	< 0.20	
Epinephrine* excretion in urine	0.093	0.457	> 0.25	< 0.15	
Total catecholamine** excretion in urine	0.047	0.453	> 0.25	< 0.15	
Aldosterone*** excretion in urine	0.024	0.187	> 0.25		
Creatinine clearance	0.018	0.321	> 0.25		

LV: Infusion of isoproterenol. **Two 12 h urine collections. ***One 24 h urine collection.

during propranolol was expressed in 12 different ways. As illustrated in Table VII and VIII significant correlations to 11 initial parameters occurred only at random (5 statistically significant correlations out of 132 calculations) indicating that none of the selected parameters was useful for prediction of response.

Usefulness of the nitroglycerine test for estimation of beta-adrenergic blockade

After 4 weeks of placebo sublingual administration of nitroglycerine 0.4 mg and change to upright posture caused an increase of pulse rate from 80.3 to 115.4 beats/min, (difference 35.1 $p < 0.0005$) (Fig. 13)

As previously mentioned, intravenous infusion of isoproterenol 1 μ g/min over 3 min increased heart rate by 12.1 beats/min. The response to nitroglycerine was not significantly correlated to the effect of isoproterenol at the infusion rate of 1 μ g/min ($r = 0.351$, n.s.) However the increase of heart rate during infusion of isoproterenol at the rate of 2 μ g/min (27.6 beats/min) was significantly correlated to the nitroglycerine effect ($r = 0.510$, $p < 0.05$)

Finally the increase of heart rate during infusion of isoproterenol at the rate of 3 μ g/min (39.8 beats/min) was also significantly correlated to the effect of nitroglycerine ($r = 0.633$, $p < 0.005$)

The nitroglycerine test was not carried out after acute I V administration of propranolol due to severe postural effects in 3 of the first 4 patients.

Following two weeks of oral propranolol therapy at 160 mg daily the average increase of pulse rate due to sublingual nitroglycerine and upright posture was 21.5 beats/min ($p < 0.005$) This was inversely correlated to plasma propranolol concentration ($r = -0.518$, $p < 0.05$) The increase of pulse rate was not significantly correlated to the logarithm of plasma

propranolol concentration ($r = -0.398$, n.s.)

Following 4 weeks of oral propranolol therapy (av 235 mg/d) the average increase of pulse rate after sublingual nitroglycerine and upright posture was 17.3 beats/min ($p < 0.005$)

The change of heart rate in response to I V isoproterenol, 3 μ g/min, was 5.3 beats/min ($p < 0.05$) Again the effects of nitroglycerine and isoproterenol were significantly correlated ($r = 0.611$, $p < 0.01$) However as opposed to the results during placebo there now was a marked difference between the two manoeuvres in regard to the quantitative response (Fig. 13)

The average plasma propranolol concentration after 4 weeks of oral treatment was 123 \pm 46.1 ng/ml but this was not significantly correlated to the nitroglycerine induced increase of pulse rate ($r = -0.184$, n.s.) Nor was the logarithm of plasma propranolol concentration significantly correlated to the effect of nitroglycerine ($r = -0.010$, n.s.)

Furthermore, plasma propranolol concentration, or its logarithm, was not significantly correlated to the effect of isoproterenol infusion ($r = -0.384$, n.s. and $r = -0.386$, n.s.)

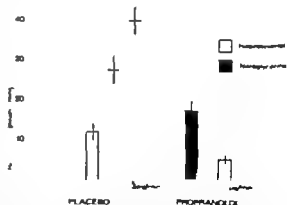


Fig. 13

Average increase of heart rate \pm S.E.M. in response to sublingual nitroglycerine 0.4 mg and assumption of upright posture and intravenous infusion of isoproterenol at the rate of 1, 2 and 3 μ g/min. Placebo indicates that the tests were performed after 4 weeks of placebo treatment. Propranolol indicates that the tests were performed after 4 weeks of oral propranolol therapy.

The effect of 10 min of 45° head-up tilt was not correlated to the effect of nitroglycerine either during placebo ($r = 0.308$, n.s.) or after 4 weeks of oral propranolol treatment ($r = 0.418$, n.s.) Nor was the effect of tilt correlated to the effect of isoproterenol either before or during propranolol therapy ($r = 0.272$, n.s. and $r = 0.238$, n.s.)

Plasma propranolol concentration

Average plasma propranolol concentration after acute I V administration of propranolol 0.22 mg/kg was 100.5 ± 6.0 ng/ml and as indicated by the S.E.M. individual variations were small. Following 2 weeks of oral propranolol at 160 mg daily the average plasma concentration was 68.3 ± 11.5 ng/ml and finally after 4 weeks of oral therapy average plasma propranolol concentration was 123.1 ± 46.1 ng/ml. It has already been indicated that the reduction of systolic blood pressure in the clinic was significantly correlated to plasma propranolol concentration after 4 weeks of oral therapy. However the degree of beta adrenergic blockade was not directly corre-

lated to plasma propranolol levels, as indicated by the response to isoproterenol infusions.

Patients receiving propranolol 160 mg daily during 4 weeks had an average plasma propranolol concentration of 68.0 ± 7.1 ng/ml after two weeks and 69.1 ± 17.0 ng/ml after 4 weeks. As expected, plasma propranolol concentration rose in the patients given 320 mg daily during the last 2 weeks. Average plasma propranolol concentration in this group after 4 weeks was 162.7 ± 82.6 ng/ml (Fig. 5). Due to the wide individual variations this change did not achieve statistical significance.

In summary plasma propranolol concentration at the end of 4 weeks of oral propranolol therapy was significantly correlated to the over all reduction of systolic recumbent and standing blood pressure as measured in the clinic. Except for this, plasma propranolol concentration was not significantly correlated to changes of hemodynamic parameters or plasma renin activity. Nor was the degree of beta-adrenergic blockade, as estimated by heart rate response to infusion of isoproterenol or sublingual nitroglycerine, correlated to plasma propranolol concentrations.

SIDE EFFECTS

Only one patient had side effects from the treatment with propranolol. Patient No. 9 complained of insomnia, being able to sleep only a few hours every night. He also experienced tiredness during the day possibly secondary to the lack of sleep. For this reason he was offered a change of therapy but volunteered to continue propranolol treatment. Subjectively some improvement occurred during continued treatment. This patient was clearly normotensive at the end of the study having a recumbent blood pressure of 115/80 mm Hg in the clinic.

With this exception, no side effects were

reported or observed. No consistent changes of weight were observed nor were there any signs of development of latent or manifest cardiac decompensation. Heart size or pulmonary venous pattern did not change radiographically. EKG changes were confined to the expected reduction of heart rate.

Repeated studies of hematological tests, renal and liver function tests as well as a number of other blood and urinary tests did not reveal any consistent changes.

As expected, postural hypotension or hypotensive symptoms during physical activity did not occur.

Antihypertensive effect

The antihypertensive effect observed in the present study is in agreement with previously published reports in this field^(25, 31, 31) indicating that beta-adrenergic blockade with propranolol in hypertensive patients is effective in reducing arterial pressure with few or no side effects and no postural hypotension. Contrary to some reports,^(25, 31) the antihypertensive action appeared to be of rapid onset as judged from the daily measurements of home blood pressure. This may be a result of the relatively high dosage (40 mg 4 times daily) that was used already from the first day of active treatment.

It is entirely possible that the dosages and the duration of treatment employed in the present study have not produced the optimal antihypertensive effect obtainable in every individual patient. However based on the results in this series of patients with mild to moderately severe essential hypertension, it can be concluded that propranolol has a useful antihypertensive action.

Hemodynamic effects

The antihypertensive effect of propranolol may seem surprising in view of the fact that the drug fails to reduce arterial pressure after acute intravenous administration⁽⁷⁸⁾ a finding that is confirmed in this study. It has been shown that when given acutely propranolol reduces cardiac output mainly through a reduction of heart rate⁽⁷⁸⁾ and this is also demonstrated in the present series. In the acute situation, total

peripheral resistance is increased in response to the reduction of cardiac output and consequently arterial pressure remains unchanged. It has been postulated that the antihypertensive effect seen during chronic propranolol therapy must be attributed to prolonged reduction of cardiac output⁽²⁰⁾. As shown in the present series, cardiac output remains at a lower level during oral propranolol therapy but total peripheral resistance is readjusted to the initial level thereby reducing blood pressure.

It has even been shown in a recent study that total peripheral resistance may fall below the initial level during long-term therapy⁽⁸⁰⁾. The mechanism by which this re-adjustment of total peripheral resistance occurs is not fully understood. Initially it was postulated that a resetting of baroreceptor sensitivity took place^(80, 81). However others rejected this hypothesis based on the erroneous assumption that total peripheral resistance remained elevated during chronic propranolol therapy⁽⁸⁰⁾.

The demonstration that peripheral resistance is re-adjusted to the initial level - or lower - makes it theoretically feasible that baroreceptors are reset. In fact, the data presented in the present study suggest that such a resetting towards a greater baroreceptor sensitivity takes place. It should be pointed out, though, that the studies on baroreceptor sensitivity as performed in the present study are concerned only with the effect on heart rate in response to a temporary increase of blood pressure. There is no reason to believe that a resetting of baroreceptor sensitivity would affect the regulation of heart rate only and

leave vasomotor control unaffected. The baroreceptor reflex was not assessed after acute beta-adrenergic blockade. However others have reported some increase of baroreceptor sensitivity after I.V. propranolol but a total abolishment of the reflex after atropine.⁽⁸²⁾ This would indicate that the reduction of heart rate in response to an acute elevation of blood pressure is effected mainly by increased vagal stimulation and not by withdrawal of beta-adrenergic stimulation. It therefore would appear to be justified to compare the baroreceptor sensitivity during placebo treatment to that during prolonged propranolol therapy. Furthermore, the findings in the present study do not demonstrate a statistically significant change of baroreceptor sensitivity probably due to the fact that only 7 pairs of observations were used.

If however baroreceptors were reset, the explanation for this is not immediately evident as blood pressure is not reduced after acute beta-adrenergic blockade. It is possible that propranolol would reduce the cardiac component of transient blood pressure peaks occurring throughout the day and thereby cause a reduction of average blood pressure during the day. This could result in a subsequent resetting of baroreceptor sensitivity. The importance of changes of flow characteristics during propranolol therapy and the prolongation of diastole due to the reduction of heart rate remains speculative in regard to its importance for baroreceptor adjustments.

It is also conceivable that central nervous effects of propranolol may be responsible for a modified efferent outflow from the vasomotor center e.g. in response to afferent baroreceptor impulses, resulting in a reduction of peripheral vascular resistance. Undoubtedly further research is needed before we understand the exact mechanism underlying the demonstrated re-adjustment of peripheral resistance.

As previously mentioned, the local anesthetic effect of propranolol obviously plays a minor role in the antihypertensive action as indicated by the lack of hypotensive effect of d. propranolol.⁽⁷⁵⁾ Furthermore, if lowering of blood pressure were to result from anesthesia of vasoconstrictor fibres, it would be expected that acute intravenous administration of propranolol should not elevate TPR. In the present series all patients but three showed a reduction of intra-arterially measured mean pressure, due to a significant difference in total peripheral resistance. However the importance of this finding is doubtful as the reduction of clinic and home blood pressures were of the same magnitude in these 3 patients as in the remaining group. It is conceivable that the antihypertensive effect was masked in the hemodynamic situation, e.g. due to anxiety.

The effect of tilt

Tilt during placebo produced a reduction of cardiac output and stroke volumes of the same order that has been reported previously in normal subjects and patients with borderline hypertension.⁽⁸⁴⁾ Similar changes were seen after intravenous and oral propranolol therapy. However the reflex increase of heart rate was abolished after acute beta-adrenergic blockade and reduced after prolonged oral propranolol therapy. The change of cardiac output due to tilt was of the same magnitude after intravenous propranolol as during placebo. However as this change started from an already reduced level of cardiac output after acute beta-adrenergic blockade, dizziness and other signs of insufficient cerebral blood flow occurred. As mentioned elsewhere heart rate response to tilt was not a useful indicator of the degree of beta-adrenergic blockade.

Effect on plasma renin activity

The mechanisms underlying variations of renin secretion are not fully known and several theories including the existence of intrarenal baroreceptors, volume receptors and chemoreceptors have been suggested. However after the demonstration of an inhibitory influence of several anti-adrenergic drugs^(41-44, 60) interest has been directed to the adrenergic mechanisms involved in renin secretion. In the present study acute beta-adrenergic blockade reduced the effect of tilt on plasma renin activity. However the effect of 4 weeks of oral propranolol therapy was much more striking, resulting in reductions of PRA to low or very low levels in all patients. It would appear therefore, that renin release is mediated largely through beta-adrenergic receptors.

In the present series of essential hypertensives there was no correlation between reduction of PRA and reduction of blood pressure. Nor was the initial level of PRA related to the response to propranolol. This is in contrast to a recent report by Bühler et al. in which patients with high PRA were shown to have a greater reduction of diastolic blood pressure during propranolol therapy than patients with low PRA.⁽⁶⁾ However as Bühler's series included patients with renal and malignant hypertension, and as no patient in the present series had high PRA no direct comparisons can be made.

Recently it has been shown that marked reductions of PRA takes place after only 3 days of oral propranolol therapy while little or no effect on blood pressure occurs.⁽⁴⁰⁾ Others have stated that blood pressure response to propranolol can not be ascribed in the majority of patients to differences in its effect on PRA.⁽⁶⁰⁾ This is in agreement with the results of the present study.

With regard to the previously discussed mechanisms underlying the re-adjustment of peripheral vascular resistance it could be argued

from a theoretical point of view that the reduction of PRA - and consequently of angiotensin - might reduce peripheral vasoconstriction and thereby lower TPR. However as PRA usually is not elevated in patients with benign essential hypertension - including the patients of the present series - the increased vascular resistance in this form of hypertension is most likely independent of this mechanism. This is further supported by the results of the present study in which no significant correlation was seen between reduction of PRA and reduction of total peripheral resistance.

The conclusion therefore must be that the reduction of PRA and the reduction of blood pressure (and peripheral resistance) in essential hypertension during propranolol therapy seem to be two separate phenomena that are not directly linked together. If low PRA constitutes a protection against cardiovascular risks, e.g. myocardial infarction or stroke as indicated in a recent study⁽⁶⁾ and if drug induced reduction of PRA will reduce such risks cannot be commented upon at the present time. Clearly this field deserves further research.

Observations on predictability

There is always a desire in medical therapy to make predictions regarding the response to therapy or even to select patients based on predictive judgement. In the present study correlations were made between the reduction of blood pressure and 11 initial parameters for the purpose of identifying factors that might help predict the response to propranolol. From a logical point of view some of the selected parameters could be expected to serve a useful purpose in this respect.

Obviously initial heart rate, cardiac output or cardiac index would seem to be factors worth studying. However hypertensive patients with high cardiac output have not responded more favourably to propranolol in previously published studies^(2, 60) and the the

results of the present study confirm this. It would seem appropriate therefore to state that administration of propranolol to hypertensive patients should not be based on whether these patients have hyperkinetic or normokinetic circulation.

Another seemingly logical factor to test would be the response to beta-adrenergic stimulation prior to administration of beta-adrenergic blockade. However the heart rate response to intravenous administration of isoproterenol was not significantly correlated to the reduction of blood pressure during propranolol therapy.

The lack of correlation between urinary catecholamine excretion and response to propranolol may not be all that surprising in view of the fact that increased excretion of norepinephrine is not a characteristic finding in essential hypertension⁽¹⁸⁾. On the other hand it has recently been shown that patients with essential hypertension have increased levels of plasma catecholamines^(19, 24) and it should be stressed that simply studying the urinary excretion of these substances may be too crude to evaluate their importance in essential hypertension.

Finally the lack of correlation between blood pressure reduction and the other parameters (age, weight, total peripheral resistance, aldosterone excretion and creatinine clearance) only leads to the conclusion that none of the employed parameters served the desired purpose of predicting the antihypertensive response to propranolol.

Estimation of beta-adrenergic blockade

Logically the way to test beta-adrenergic blockade is by using a beta-adrenergic stimulant, e.g. isoproterenol. As this involves an intravenous infusion there has been a search for simpler methods. After employing a variety of tests e.g. physical exercise and hyperventilation, Fitzgerald concluded that registration of heart rate after sublingual administration of

nitroglycerine was best suited as it was least affected by atropinization⁽¹⁹⁾. In the present study the nitroglycerine test has been employed in a different fashion from Fitzgerald's original description⁽¹⁷⁾ as the patients assumed upright posture after the administration of nitroglycerine. In the initial untreated state there was a good correlation between the response to nitroglycerine and the tachycardia produced by intravenous infusion of isoproterenol 3 µg/min. Both manoeuvres produced almost identical increase of heart rate in this situation. However during oral therapy with propranolol the increase of heart rate due to nitroglycerine was three times greater in spite of a significant correlation to the effect of isoproterenol. Naturally this reduces the usefulness of the nitroglycerine test as it indicates that the test may not be entirely specific. The greater increase of heart rate due to nitroglycerine may indicate that vagal withdrawal affected the response. Whether this difference is due to the fact that isoproterenol exerts its main effect on the resistance vessels whereas nitroglycerine is comparatively more effective on capacitance vessels⁽²⁰⁾ can not be answered by the present study. From a practical point of view the discrepancy of heart rate response to isoproterenol and nitroglycerine during propranolol therapy indicates that the nitroglycerine test, as employed in the present study can only be used for crude estimations of the degree of beta-adrenergic blockade.

The lack of correlation between the increase of heart rate during tilt and the response to isoproterenol and nitroglycerine indicated that tilt is not a useful manoeuvre for estimation of beta-adrenergic blockade.

Finally in view of recent reports regarding the lack of correlation between plasma propranolol and the degree of beta-adrenergic blockade⁽²¹⁾ it is not surprising that the response to isoproterenol or nitroglycerine was not significantly correlated to plasma propranolol concentration.

Plasma propranolol concentration

Plasma propranolol concentration at the end of 4 weeks of oral treatment was significantly correlated to the reduction of systolic blood pressure both in the recumbent and standing position, while the overall reduction of diastolic blood pressure was not correlated to plasma propranolol concentration.

More interesting is the observation that once a certain reduction of blood pressure had occurred - as observed after 2 weeks of oral therapy in the present study - further reductions of both recumbent and standing systolic and diastolic blood pressure were directly linked to increasing plasma propranolol concentrations. This would indicate that increasing antihypertensive effect may be expected from increasing plasma propranolol concentrations. Obviously wide individual variations of

absorption must be taken into consideration but this phenomenon could explain the impressive antihypertensive effect of propranolol reported in series where comparatively large doses were given ^(81 82)

As mentioned previously the lack of correlation between plasma propranolol concentration and the degree of beta-adrenergic blockade, as estimated by the response to intravenous isoproterenol is in agreement with recent observations by others ⁽⁸³⁾ This could indicate that tissue binding of propranolol is a stronger determinant of the degree of beta-adrenergic blockade than plasma concentrations. Another possibility is that metabolites with beta-blocking capacity e.g. 4-hydroxy propranolol contribute significantly to the degree of blockade thereby explaining the lack of correlation to plasma propranolol concentration.

CONCLUSIONS

Oral therapy with propranolol in essential hypertension caused significant reductions of systolic and diastolic blood pressure already during the first week of therapy. The dosage used - 160 to 320 mg daily - caused a significant degree of beta-adrenergic blockade as demonstrated by the response to intravenous infusions of isoproterenol.

The reduction of systolic blood pressure was significantly correlated to plasma propranolol concentration. Hemodynamically acute administration of propranolol intravenously caused a significant reduction of cardiac output but no decrease of blood pressure due to an increase of systemic vascular resistance. During prolonged oral therapy with propranolol cardiac output remained significantly reduced but peripheral resistance was re-adjusted towards the initial level resulting in a decrease of arterial pressure.

The mechanisms underlying this adjustment of systemic vascular resistance may be secondary to a resetting of baroreceptors.

Reduction of plasma renin activity during prolonged oral treatment with propranolol is impressive. However a direct connection between the reduction of PRA and arterial pressure could not be demonstrated.

It was not possible to predict the antihypertensive response to propranolol in individual patients by studying parameters such as heart rate or cardiac output in the initial untreated state.

Finally heart rate response to sublingual nitroglycerine and assumption of upright posture was found to give only a crude estimation of the degree of beta-adrenergic blockade as tested by intravenous isoproterenol infusion.

GENERAL SUMMARY

This study has been concerned with the effects of acute and prolonged beta-adrenergic blockade with propranolol in essential hypertension. Fifteen male patients, all with mild to moderately severe essential hypertension, were included in the study. Initially placebo was given (single-blind) as the only treatment for 4 weeks. Hemodynamic parameters including cardiac output (dye dilution technique) and plasma renin activity were studied at the end of the placebo period. Propranolol was then given intravenously 0.22 mg/kg of body weight, and the studies of hemodynamics and renin were repeated. Following acute beta-adrenergic blockade there was a significant reduction of cardiac output with a compensatory increase of total peripheral resistance resulting in unchanged arterial pressure. The effect of tilt on plasma renin activity was diminished after acute beta-adrenergic blockade.

During oral treatment with propranolol significant reductions of systolic and diastolic blood pressure occurred already during the first week of therapy as judged from daily measurements of home blood pressure. The over all effect of 4 weeks of oral propranolol therapy on clinic blood pressure was a reduction of recumbent systolic pressure by 28.6 mm Hg ($p < 0.0001$) and of diastolic pressure by 19.9 mm Hg ($p < 0.0001$). Significant reductions of home blood pressure were seen as well.

Repeated hemodynamic investigations after 4 weeks of oral propranolol therapy revealed that cardiac output was still significantly reduced whereas systemic vascular resistance was re-adjusted to the initial level thereby re-

sulting in a reduction of arterial pressure. Studies of the baroreceptor reflex suggested a resetting towards a greater sensitivity.

A marked decrease of plasma renin activity was seen after 4 weeks of oral propranolol therapy indicating that beta-adrenergic mechanisms play an important role in the mediation of renin release. The reduction of renin was not significantly correlated to the reduction of blood pressure indicating that the antihypertensive effect of propranolol in mild essential hypertension is not directly dependant on changes of plasma renin activity.

Attempts to define parameters of predictive value for the antihypertensive response to propranolol were not successful. No correlation was found between factors such as initial heart rate, cardiac output or the response to beta-adrenergic stimulation and the reduction of blood pressure.

Studies of heart rate after sublingual administration of nitroglycerine and assumption of upright posture indicated that this will provide only a crude estimation of the degree of beta-adrenergic blockade.

Plasma propranolol concentration was found to be significantly correlated to the reduction of systolic blood pressure both in the recumbent and standing position whereas the change of diastolic blood pressure was not. On the whole, plasma propranolol concentration proved to be of little value as not even the degree of beta-adrenergic blockade, as assessed with intravenous isoproterenol infusion, was significantly correlated to it.

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